MIXING DESIGNS: A GENERAL MODEL, SIMPLIFICATIONS, AND SOME MINIMAL DESIGNS

by

David B. Hall and W. T. Federer

May, 1976

Abstract

Many different models have been proposed for experimental data on mixtures, single units being subjected to a number of agents. Among these, one which has proved useful in explaining and analyzing the data of genetics and agricultural mixtures is the general and specific combining ability model. A similar model has been proposed for use in chemical experimentation (see Free and Wilson, 1964). The success of this model in the specialized areas where it has been applied invites the consideration of some generalization of it whenever a problem of mixtures is to be attacked and the experimenter is looking at his alternatives in terms of models. This paper contains the construction of a general model which extends the concepts of general and specific combining ability.

Parameters are defined as deviations from expectations and can be meaningfully interpreted. The method of definition puts natural constraints on the parameters, leading to their estimability.

In specific experimental situations, a simplification of the general model will usually be appropriate. Having simplified the model, it becomes possible to consider designs involving less treatments than the full model requires. Designs are discussed in this paper which allow estimability of parameters for some simplifications of the general model and which involve as few treatments as possible.

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A Thesis
Presented to the Faculty of the Graduate School of Cornell University for the Degree of Master of Science

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David Benjamin Hall
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Biographical Sketch

David Benjamin Hall was born on January 17, 1951 in Ithaca, New York. His education has been in the Seneca Falls public school system and at Colgate University. He received a Bachelor of Arts Degree from Colgate in Mathematics in 1973. Since that time he has been studying with the Biometrics Unit of the Department of Plant Breeding and Biometry, Cornell University, with philosophy as his minor field. He does not like writing about himself.
Acknowledgments

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I. Introduction

It is a common situation in experimentation to have a number of contributing agents applied to each experimental unit and then make one observation from each experimental unit. When a group of the contributing agents are elements of the same population, the treatment applied to the experimental unit involves a mixture of elements from that well-defined population. A simple statistical design for mixtures would be a design where all the contributing agents being considered are elements of one population.

The general class of simple statistical designs for mixtures, as defined above, is unmanageably large when it comes to considering general models and good characteristics for treatment designs. Three subclasses recommend themselves immediately. When the amount of a contributing agent applied to an experimental unit is allowed to vary over a continuum, some sort of regression model is appropriate. When the amount of a contributing agent applied to an experimental unit has fixed, discrete levels and any level of one agent may occur with any combination of levels of the other agents in the population of contributing agents, the arrangement is a factorial one. A third possibility is that a fixed size contribution from the population of contributing agents is to be applied, and if k contributing agents are applied to an experimental unit an equal proportion of the total contribution will come from each agent.

The subclass of the general class of statistical designs for mixtures which calls for a regression model has been studied extensively as part of response surface methodology (see Cornell, 1973 and Mead and Pike, 1975). The subclass of the general design which involves factorial arrangements is also well presented in existing literature (see Yates, 1937, and Federer and Balaam, 1972). The third
subclass has been considered in medical and genetic research and has been con-
sidered as potentially generalizable and applicable in a wide range of scientific fields (see Federer, 1975, and Federer and Hall, 1975).

In the field of genetics it is necessary, in general, to speak of mixtures of size two. The biologist looking at any animal or plant which reproduces sexually must look at offspring as the product of a genetic mixture of the two parents. Thus, the offspring is the experimental unit with the parents as the contributing agents being applied. Moving into other fields, where nature allows more than two agents to contribute to one experimental unit, the model from genetics must be generalized.

The concepts to be borrowed from genetics and generalized to fit a more general model are the concepts of general combining ability and specific combining ability (see Henderson, 1952, and Kempthorne, 1957).

"By general combining ability we mean the average merit with respect to some trait or weighted combination of traits of an indefinitely large number of progeny of an individual or line when mated with a random sample from some specified popu-
lation."

"General combining ability has no meaning unless its value is considered in relationship to at least one other individual or line and unless the tester popu-
lation and the environment are specified."

"We shall define specific combining ability as the deviation of the average of an indefinitely large number of progeny of two individuals or lines from the values which would be expected on the basis of the known general combining abilities of these two lines or individuals ...".*

In order to distinguish between the combining abilities already defined for genetics and the generalizations of these concepts to be dealt with by this paper, the generalization of effect due to general combining ability will be called general mixing effect and the generalizations of effect due to specific combining ability will be called specific mixing effects.

In chemistry, a mathematical model for explaining biological response data has been proposed which involves a term comparable to the general combining ability term in genetics models (see Free and Wilson, 1964). Several organic compounds which differ from each other due to having different substituents at a number of locations in the molecule are tested to yield biological response. The model for the observed response of a specific compound is the mean of all compounds in the population of potential compounds plus an effect attributable to each of the substituents which is at a location in the molecule where the population contains at least one molecule with a different substituent at that location. The effect which is due to the substituent at a location where the substituent is allowed to vary is comparable to the general combining ability in the genetic model.

A simple example of a situation where this model might be applied would be the consideration of the following molecule and the biological response when it is applied as an analgesic. Consider

\[
\begin{align*}
\text{NHCOCHR}_2 \\
\text{R}_1 \\
\text{C}_6\text{H}_5
\end{align*}
\]

\(\text{R}_1\) may be either H or CH\(_3\). \(\text{R}_2\) may be either N(CH\(_3\))\(_2\) or N(C\(_2\)H\(_5\))\(_2\). The model for the response for a particular compound would involve a term for the overall average response, a term for the contribution of the substituent at location \(\text{R}_1\), and a term for the contribution of the substituent at location \(\text{R}_2\).
In agriculture there has been interest in growing mixtures involving lines of 
*M. sativa*, *Trifolium pratense*, *Sojamax*, *Avena sativa*, and other cultivars. To test
for the beneficial or detrimental effects of using mixtures as compared with using
solid seeding of a single strain of cultivar, a model based on general and specific
mixing effects has been proposed (Federer, 1975). A model for the yield of a field
which has been planted with a mixture of seeds has been proposed, containing com-
ponents defined as general mixing effects and specific mixing effects. To general-
ize this model is the first aim of this paper.

The foundations for the work in this paper are laid in section two. Defi-
nitions, notation, and concepts are presented. In section three a general model is
proposed for mixing designs for which regression models and factorial models are
inappropriate. Parameters, constraints on the parameters, and estimability are
discussed.

The general model is too general for most practical situations. In section
four several situations when the model might be simplified are discussed. Section
five is the consideration of a specific class of simplified models and minimal
treatment designs for estimating all parameters. Theory from balanced incomplete
block designs (BIB's) is used. An example from the class of models considered in
section five is presented in section six. The last section is an appendix contain-
ing some minimal treatment designs.
II. Foundations

II.1. Concepts

The experimental situation of interest involves objects (i.e., fields, animals, chemical compounds, etc.) subjected to several agents (i.e., varieties of crops, drugs, radicals, etc.), all of which make some contribution to the observed value for each object (i.e., yield, weight, rate of reaction, etc.). If an object is subjected to a particular agent, the amount of that agent applied to that object is a function of \( n \), the number of different agents applied to the object in question.

Two functions of the number of agents applied should be mentioned as the most likely to be encountered. If the total amount of treatment applied to each object is to be held constant, then the amount of an agent to be applied will be such that all agents applied contribute equally. The amount of an agent applied when there are \( m \) agents applied to the object is \( m^{-1} \) times the total amount of treatment. The other situation is when the amount of an agent to be applied is a constant function of the number of agents applied. This is the case when each agent may either be applied at only one level or not applied at all.

The genetic arrangement described in the introduction is a situation where the amount of agent applied must be constant. The number of agents (parents) applied to a particular object (progeny) must be constant at two. In the chemical arrangement, as described in the introduction, the number of agents applied to a particular object must be held constant throughout the experiment. In the specific chemical example mentioned, two is the number of agents to be applied, so the model is the same as the model in the genetics arrangement.
In the agricultural example, the amount of a cultivar variety to be applied to a field is a non-constant function of \( n \) because the total amount of seed planted should be held constant. This means that if \( n = 4 \), each variety applied to a field will make up one-fourth of the total application. If \( n = 2 \), each variety applied will make up one-half of the total application. Each level of \( n \) considered, adds another level to each of the varieties. Since, for all varieties the level applied is a function of the number of varieties applied, not all combinations of levels and varieties are possible.

II.2. Definitions

In genetic diallel cross models two agents, parents, contribute to each object, progeny. The simple model is explained by defining two effects, general combining ability and specific combining ability. When the number of agents allowed to act on an object is varied, the number of definitions necessary to explain the system multiplies at an increasing rate.

If situations involving pure strains, the application of only one agent to each object, are considered in addition to applications of two or more agents, then we define a new factor and modify the geneticists' definitions. The effect of an agent when applied by itself should be a measure of the "pure" effect of that agent. Letting the terminology involving mixtures precede any mixing, mono-specific mixing effect will be defined as this "pure" effect. The general effect of an agent when applied in mixtures of size two will be comprised of two components. One will be the mono-specific mixing effect just defined. The other component is a general effect due to having mixtures of size two instead of pure strains. (1)-General mixing effect will be defined as this general effect due to having mixtures of size two or greater. Bi-specific mixing effect is defined as the deviation of the mean effect of a specific pair of agents from the values which
would be expected on the basis of the general effects; i.e., mono-specific mixing effects and general mixing effects.

Now, consider the experimental situation when the arrangements of agents to be considered include the two above and mixtures of size three. The general effect of an agent when three agents are applied will be composed of the mono-specific mixing effect, the (1)-general mixing effect, and a deviation from the sum of these two effects. (2)-General mixing effect will be defined as the deviation of the average effect of an agent in the presence of two other agents from the effect expected on the basis of the mono-specific mixing effect and the (1)-general mixing effect. Similarly, (1)-bi-specific mixing effect will be defined as the deviation of the average effect due to the presence of a specific pair of agents with one other agent from the effect expected on the basis of the general effects of the two agents and the bi-specific mixing effect. Tri-specific mixing effect is defined as the deviation of the average effect of an application of a specific set of three agents from the effect expected on the basis of all the effects previously defined.

To generalize and extend these definitions, consider the experimental situation where mixtures of size k are to be applied in addition to all mixtures of size less than k. The k-general mixing effect of an agent is the deviation of the average effect of that agent when k agents are applied from the average effect of that agent when (k-1) agents are in a treatment. For $r = 2, 3, \ldots, k-1$, the $(k-r)$-r-specific mixing effect is the deviation of the average effect due to having a specific set of r agents among the k agents applied from the average effect which would be expected based on knowledge of the average effects of the r different sets of r-1 agents which are subsets of the specific set of r agents and knowledge of the r-specific mixing effect, the (1)-r-specific mixing effect, the (2)-r-specific mixing effect, up to the $(k-r+1)$-r-specific mixing effect. The k-specific mixing
effect is defined as the deviation of the average effect of the particular mixture of k agents from the effect which would be predicted based on knowledge of the average effects of the k different sets of k-1 agents which are subsets of the set of k agents which is under consideration.

These definitions partition the effect due to treatment with a particular mixture of k agents into components for the general effect of each agent, the additional general effect of each pair of agents, the additional general effect of each triplet of agents, up to the additional general effect of the complete set of k agents. The general effect of each agent is further partitioned into mono-specific mixing effect and (1) through (k-1)-general mixing effect. The additional general effect due to each pair of agents is partitioned into bi-specific mixing effects and (1) through (k-2)-bi-specific mixing effects. The additional general effect of each set of r agents for any value of r less than k is partitioned into r-specific mixing effects and (1) through (k-r)-r-specific mixing effects.

II.3. Notation

The observation yielded by an object in the sample will be denoted \( Y_{(n)ij} \) in the general case. The \( n \) stands for the number of agents applied to the object. The \( i \) is the number of the replicate of the treatment which this object is. The subscript \( j \) will indicate which of the possible specific mixtures of size \( n \) has been applied to the object. Thus \( n \) and \( j \) specify the treatment of the object and \( i \) specifies which replicate of that treatment.

The number of agents applied in a treatment may have an effect independent of which agents are applied. To account for this, there will be a mean specific to the number of agents in a treatment. The mean for treatments containing \( n \) agents will be denoted \( \mu_{(n)} \).
The effect of replicate $i$ would be denoted $\rho_i$. The common treatment of this effect would be to assume it was normally distributed with mean zero and variance $\sigma^2$. The replicate effects observed are assumed to be independently and identically distributed (IID). The error term for the object whose yield is subscripted $(n)ij$ will be denoted $\varepsilon(n)ij$ and the observed values are assumed to be IID normal with mean zero and variance $\sigma^2$.

The mono-specific mixing effect for agent $t$ will be denoted $\tau_t$. The (1)-general mixing effect for agent $t$ will be denoted $(1-\alpha)_t$. The (k)-general mixing effect for agent $t$ will be denoted $(k-\alpha)_t$. For a particular combination of $r$ agents, the $r$-specific mixing effect will be represented by $\beta$ subscripted with the numbers of the appropriate $r$ agents. The $(k-r)$-$r$-specific mixing effect will be represented by $((k-r)\beta)$ subscripted with the numbers of the appropriate $r$ agents.

To eliminate ambiguity concerning what agents went into the treatment of an object $Y(n)j$, the following system will be used:

The $v$ agents being applied in the experiment under consideration are labeled with the numbers 1, 2, $\cdots$, $v$. The $\binom{v}{n}$ possible combinations of $n$ agents are completely ordered in the following way. Each combination is written as

$$(i_1, i_2, \cdots, i_n)$$

where $i_h$ is the number representing one of the $n$ agents in the combination. The $i_h$'s are ordered; that is, if $h < h'$ then $i_h < i_{h'}$. Considering any two combinations $I = (i_1, i_2, \cdots, i_n)$ and $J = (j_1, j_2, \cdots, j_n)$ then $I < J$ if

(i) $i_1 = j_1$, $i_2 = j_2$, $\cdots$, $i_m = j_m$ for some $m < n$ ($m = 0, 1, 2, \cdots, n-1$);

(ii) $i_{m+1} < j_{m+1}$.

This provides a complete ordering on the set of combinations of size $n$. These combinations are now labeled with consecutive positive integers starting with one, and this label will correspond to the $j$ in $Y(n)j$.
For example, when \( v = 7 \) and \( n = 3 \) the \( \binom{7}{3} = 35 \) combinations are ordered as follows:

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So \( Y_{(3)\cdot 17} \) is an observation from an object treated with a mixture of agents 2, 3, and 5.

An indicator function will be of use in writing out general model equations. The function will be denoted:

\[
I_{nj}(h_k) = \begin{cases} 
1 & \text{if agent } h_k \text{ is in the } j^{th} \text{ combination of } n \text{ agents} \\
0 & \text{otherwise.}
\end{cases}
\]

\( h_k = 1, 2, \ldots, v \);
\( k = 1, 2, \ldots, n \).
III. The Model

III.1. The General Model

The definitions and notations in the previous section describe the model in terms of the elements which it is to contain. The model is additive, but because of the possible dependence of the amount of an agent applied on the number of agents applied it is not possible to keep the coefficients of all the parameters at one or zero in every yield equation.

The definitions are most simply interpretable if the effects are seen as linear functions of the amount of the agents applied. This means that when the number of agents applied is allowed to vary and the total amount of treatment is held constant the sum of the coefficients of effects of the same type will be one. For example, a $Y_{(3)i}^j$ yield equation will contain three terms of the form $\beta_{h_1 h_2}$ so each term will have coefficient $\frac{1}{3}$. A $Y_{(7)i}^j$ yield equation will contain $\binom{7}{2} = 21$ terms of the form $\beta_{h_1 h_2}$ so each will have the coefficient $1/21$.

The model equations will be written here with coefficients appropriate to fixed total amount of treatment and variable number of agents applied. When adapting the formula to the case where the amount of each agent applied is either zero or a constant value, it is both convenient and more meaningful that all positive coefficients should be one.

For observations on objects to which one agent has been applied, the model equation is:

$$Y_{(1)i}^j = \mu_{(1)} + r_i + \sum_{h_1=1}^{v} n_j(h_1) \tau_{h_1} + \epsilon_{(1)i}^j \quad i = 1, 2, \cdots, r; \quad j = 1, 2, \cdots, v.$$ 

The number of agents in the design is $v$. There are $r$ replicates. The model
equation contains an overall average \( \mu(1) \), a replicate effect \( \rho_1 \sim N(0, \sigma^2_r) \), a treatment effect \( \tau_{h_1} \) for some \( h_1 \), and an error term \( \epsilon_{(1)ij} \sim N(0, \sigma^2_e) \).

When two agents have been applied to the observational unit, the model equation is:

\[
Y_{(2)ij} = \mu(2) + \rho_1 + \sum_{h_1=1}^{v} I_{2j}(h_1) \left( \frac{\tau_{h_1}}{2} + \frac{(1-\alpha)_{h_1}}{2} \right) + \sum_{h_2=h_1+1}^{v} I_{2j}(h_2)\beta_{h_1 h_2} + \epsilon_{(2)ij} \\
i = 1, 2, \ldots, r \quad \text{and} \quad j = 1, 2, \ldots, \binom{v}{2}.
\]

The model contains an overall average for mixtures of two agents \( \mu(2) \), a replicate effect \( \rho_1 \), two treatment effects of the form \( \tau_{h_1}/2 + (1-\alpha)_{h_1}/2 \), an interaction effect \( \beta_{h_1 h_2} \), and an error term \( \epsilon_{(2)ij} \). The term \( \tau_{h_1}/2 + (1-\alpha)_{h_1}/2 \) is the total general effect of applying agent \( h_1 \) as one of two agents in the treatment. This is partitioned into a component involving mono-specific mixing effect \( \tau_{h_1} \) which is present whenever \( h_1 \) is present in the mixture, and a component involving \( (1-\alpha)_{h_1} \) which is present whenever \( h_1 \) is in a mixture of two or more agents. The interaction component is what has been defined as bi-specific mixing effect when only two agents are present.

Continuing to mixtures of three agents, the model equation is:

\[
Y_{(3)ij} = \mu(3) + \rho_1 + \sum_{h_1=1}^{v} I_{3j}(h_1) \left( \frac{3\tau_{h_1}}{2} + \frac{3(1-\alpha)_{h_1}}{2} + \frac{3(2-\alpha)_{h_1}}{2} \right) \\
+ \sum_{h_2=h_1+1}^{v} I_{3j}(h_2) \left( \frac{2\beta_{h_1 h_2}}{3} + \frac{(1-\beta)_{h_1 h_2}}{3} \right) + \sum_{h_3=h_2+1}^{v} I_{3j}(h_3)\beta_{h_1 h_2 h_3} \\
+ \epsilon_{(3)ij} \quad i = 1, 2, \ldots, r \quad \text{and} \quad j = 1, 2, \ldots, \binom{v}{3}.
\]
The model equation contains an error term \((\epsilon (3)_{ij})\), an overall mean for mixtures of three agents \((\mu (3))\), a replicate effect \((\rho _1 )\), three treatment effects of the form \(\left( \frac{1}{3} \left[ \tau _{h_1} + (1-\alpha )_{h_1} + (2-\alpha )_{h_1} \right] \right), \) three second order interaction terms of the form \(\left( \frac{1}{3} \left[ \beta _{h_1 h_2} + (1-\beta )_{h_1 h_2} \right] \right),\) and one third order interaction term \((\beta _{h_1 h_2 h_3})\). The treatment effect terms partition into terms involving mono-specific mixing effect \((\tau _{h_1})\), (1)-general mixing effect \(( (1-\alpha )_{h_1})\), and (2)-general mixing effect \(( (2-\alpha )_{h_1})\) which is present whenever \(h_1\) is in a mixture of size three or greater. The second order interaction term partitions into a term due to bi-specific mixing effect \((\beta _{h_1 h_2})\) which is present whenever \(h_1\) and \(h_2\) are in a mixture, and a term due to (1)-bi-specific mixing effect \(( (1-\beta )_{h_1 h_2})\) which is present whenever \(h_1\) and \(h_2\) are in a mixture of at least three agents. The third order interaction is what has been defined as tri-specific mixing effect \((\beta _{h_1 h_2 h_3})\).

The general model equation for a mixture of \(n\) out of \(v\) agents is:

\[
y_{(n)ij} = \mu (n) + \rho _1 + \epsilon (n)_{ij} + \sum _{h_1 = 1}^{v} I_{n,j}(h_1) \left( \frac{1}{n} \tau _{h_1} + \frac{1}{n} (1-\alpha )_{h_1} + \frac{1}{n} (2-\alpha )_{h_1} + \ldots + \frac{1}{n} ((n-1)-\alpha )_{h_1} \right) + \sum _{h_2 = h_1 + 1}^{v} I_{n,j}(h_2) \left[ \left( \begin{array}{c} n \\ 2 \end{array} \right) \beta _{h_1 h_2} + \left( \begin{array}{c} n \\ 2 \end{array} \right) (1-\beta )_{h_1 h_2} + \ldots + \left( \begin{array}{c} n \\ 2 \end{array} \right) ((n-2)-\beta )_{h_1 h_2} \right] + \sum _{h_3 = h_2 + 1}^{v} I_{n,j}(h_3) \left[ \left( \begin{array}{c} n \\ 3 \end{array} \right) \beta _{h_1 h_2 h_3} + \left( \begin{array}{c} n \\ 3 \end{array} \right) (1-\beta )_{h_1 h_2 h_3} + \ldots + \left( \begin{array}{c} n \\ 3 \end{array} \right) ((n-3)-\beta )_{h_1 h_2 h_3} \right] + \ldots\
\]
\[
+ \sum_{n=1}^{v} I_{n_j}(h_{n-1}) [(\frac{n}{n-1})^{-1}\beta_{h_1 h_2 \cdots h_{n-1}} + (\frac{n}{n-1})(1-\beta)_{h_1 h_2 \cdots h_{n-1}}]
\]

\[
+ \sum_{n=1}^{v} I_{n_j}(h_{n})\beta_{h_1 h_2 \cdots h_{n-1} h_n} \cdots ) ) ) .
\]

\[i = 1, 2, \ldots, r; j = 1, 2, \ldots, (\frac{v}{n}); \text{and } n = 1, 2, \ldots, v.\]

The first line in this model equation includes a term for the average yield for an object treated with \(n\) agents \(\mu(n)\), a term for the effect of replicate \(\rho_1\), and an error term \(\epsilon(n)_{1j}\). The second line is made up of terms for main effects of the agents present. The third line is made up of terms for second order interactions of the form

\[\left[\left(\frac{n}{2}\right)^{-1}\beta_{h_1 h_2} + (1-\beta)_{h_1 h_2} + \cdots + (\frac{n}{n-2})(1-\beta)_{h_1 h_2}\right].\]

For \(\ell = 3, 4, \ldots, n+1\), the \(\ell^{th}\) line is made up of terms for \(\ell-1\) order interactions of the form

\[\left[\left(\frac{n}{\ell-1}\right)^{-1}\beta_{h_1 h_2 \cdots h_{\ell-1}} + (1-\beta)_{h_1 h_2 \cdots h_{\ell-1}} + \cdots + (\frac{n}{n-\ell+2})(1-\beta)_{h_1 h_2 \cdots h_{\ell-1}}\right].\]

The main effect term

\[\left[\frac{1}{E}(\tau_{h_1} + (1-\alpha)_{h_1} + \cdots + (n-1-\alpha)_{h_1})\right]\]

is the general mixing effect of agent \(h_1\) in mixtures of \(n\) agents. This is partitionable into a term which is present whenever \(h_1\) is present \(\tau_{h_1}\), and \(n-1\) terms, each representing an effect of the presence of agent \(h_1\) in a mixture of at least some number of agents between two and \(n\). For \(m = 1, 2, \cdots, n-1\), there is a term \((m-\alpha)_{h_1}\) which represents an effect of agent \(h_1\) on the yield of a mixture which
is only present when the mixture contains at least m+1 agents. This effect, 
\((\text{m-alpha})_{h_1}\), is defined as the (m)-general mixing effect of agent \(h_1\).

For \(p = 2, 3, \ldots, n-1\), there are in the model terms of the form

\[
\left[\frac{1}{n!} \left(\beta_{h_1h_2\ldots h_p} + (1-\beta)_{h_1h_2\ldots h_p} + \ldots + ((n-p)-\beta)_{h_1h_2\ldots h_p}\right)\right].
\]

This term represents the total \(p\)-specific mixing effect for the agents \(h_1 h_2 \ldots h_p\) as a subset of \(n\) agents in the mixture. This is partitionable into a term which is present whenever the agents \(h_1 h_2 \ldots h_p\) are present \((\beta_{h_1h_2\ldots h_p})\), and \(n-p\) terms, each representing an effect of the presence of the set of agents \(h_1 h_2 \ldots h_p\) in a mixture of at least some number of agents between \(p+1\) and \(n\). For \(m = 1, 2, \ldots, n-p\) there is a term \((\text{m-beta})_{h_1h_2\ldots h_p}\) which represents an effect of the specific set \(h_1 h_2 \ldots h_p\) on the yield of a mixture which is only present when the mixture contains at least \(m+p\) agents. This event, \((\text{m-beta})_{h_1h_2\ldots h_p}\), is defined as the (m)-\(p\)-specific mixing effect.

As an example, consider the yield equation for the experimental unit denoted \(Y(5)_{11}\) when there are at least five agents which are potential elements of a mixture. This is an experimental unit treated with a mixture of the first five agents and its yield equation is:

\[
Y(5)_{11} = \mu(5) + \rho_1 + \frac{1}{5}\tau_1 + (1-\alpha)_{1} + (2-\alpha)_{1} + (3-\alpha)_{1} + (4-\alpha)_{1} \\
+ \frac{1}{5}\tau_2 + (1-\alpha)_{2} + (2-\alpha)_{2} + (3-\alpha)_{2} + (4-\alpha)_{2} \\
+ \frac{1}{5}\tau_3 + (1-\alpha)_{3} + (2-\alpha)_{3} + (3-\alpha)_{3} + (4-\alpha)_{3} \\
+ \frac{1}{5}\tau_4 + (1-\alpha)_{4} + (2-\alpha)_{4} + (3-\alpha)_{4} + (4-\alpha)_{4} \\
+ \frac{1}{5}\tau_5 + (1-\alpha)_{5} + (2-\alpha)_{5} + (3-\alpha)_{5} + (4-\alpha)_{5}
\]
\[
\begin{align*}
+ \frac{1}{10} & \left( \beta_{12} + (1-\beta)_{12} + (2-\beta)_{12} + (3-\beta)_{12} \right) \\
+ \frac{1}{10} & \left( \beta_{13} + (1-\beta)_{13} + (2-\beta)_{13} + (3-\beta)_{13} \right) \\
+ \frac{1}{10} & \left( \beta_{14} + (1-\beta)_{14} + (2-\beta)_{14} + (3-\beta)_{14} \right) \\
+ \frac{1}{10} & \left( \beta_{15} + (1-\beta)_{15} + (2-\beta)_{15} + (3-\beta)_{15} \right) \\
+ \frac{1}{10} & \left( \beta_{23} + (1-\beta)_{23} + (2-\beta)_{23} + (3-\beta)_{23} \right) \\
+ \frac{1}{10} & \left( \beta_{24} + (1-\beta)_{24} + (2-\beta)_{24} + (3-\beta)_{24} \right) \\
+ \frac{1}{10} & \left( \beta_{25} + (1-\beta)_{25} + (2-\beta)_{25} + (3-\beta)_{25} \right) \\
+ \frac{1}{10} & \left( \beta_{34} + (1-\beta)_{34} + (2-\beta)_{34} + (3-\beta)_{34} \right) \\
+ \frac{1}{10} & \left( \beta_{35} + (1-\beta)_{35} + (2-\beta)_{35} + (3-\beta)_{35} \right) \\
+ \frac{1}{10} & \left( \beta_{45} + (1-\beta)_{45} + (2-\beta)_{45} + (3-\beta)_{45} \right) \\
+ \frac{1}{10} & \left( \beta_{123} + (1-\beta)_{123} + (2-\beta)_{123} \right) \\
+ \frac{1}{10} & \left( \beta_{124} + (1-\beta)_{124} + (2-\beta)_{124} \right) \\
+ \frac{1}{10} & \left( \beta_{125} + (1-\beta)_{125} + (2-\beta)_{125} \right) \\
+ \frac{1}{10} & \left( \beta_{134} + (1-\beta)_{134} + (2-\beta)_{134} \right) \\
+ \frac{1}{10} & \left( \beta_{135} + (1-\beta)_{135} + (2-\beta)_{135} \right) \\
+ \frac{1}{10} & \left( \beta_{145} + (1-\beta)_{145} + (2-\beta)_{145} \right) \\
+ \frac{1}{10} & \left( \beta_{234} + (1-\beta)_{234} + (2-\beta)_{234} \right) \\
+ \frac{1}{10} & \left( \beta_{235} + (1-\beta)_{235} + (2-\beta)_{235} \right)
\end{align*}
\]
III.2. Constraints

In order to achieve a unique solution when estimating parameters of the model, a number of constraints must be imposed. If treatments involving mixtures of size one up to mixtures of \( k \) agents are in the experimental design, the number of parameters in the model is

\[
\sum_{i=0}^{k} (k+1-i)(\binom{v}{i}) - 1.
\]

The total number of possible treatment combinations is \( \sum_{i=1}^{k} \binom{v}{i} \).

Begin with the restriction that \( k \leq \frac{v}{2} \) that the largest mixture to be considered contains no more than half of the agents under consideration. The reason for this restriction will become apparent later on.

Constraints which do not alter the meaning of linear contrasts of effects are:

\[
\tau_i \left( \sum_{i=1}^{v} \tau_i \right) = 0, \quad (q-\alpha)_i = \sum_{i=1}^{v} (q-\alpha)_i = 0 \text{ for all } q = 1, 2, \ldots, k-1.
\]

\[
\beta_{h_1} = \sum_{j=1}^{v} \beta_{h_1 h_j} = 0 \text{ and } (q-\beta)_h = \sum_{j=1}^{v} (q-\beta)_{h_j h_j} = 0 \text{ for all } q = 1, 2, \ldots, k-2 \text{ and all } h_1 = 1, 2, \ldots, v.
\]
$\beta_{h_1 h_j} = \sum_{l=1, l \neq 1, l \neq j}^{v} \beta_{h_1 h_l h_j} = 0$ and $(q-\beta)_{h_1 h_j} = \sum_{l=1}^{v} (q-\beta)_{h_1 h_l h_j} = 0$

for all $q = 1, 2, \ldots, k-3$

and all possible pairs $h_1 h_j$.

\[ (1-\beta)_{h_1 h_2 \cdots h_{k-2}} = 0 \]

for all possible mixtures of $k-2$ agents.

Each mixture of level $m$ adds $\sum_{i=0}^{m} \binom{Y}{1}$ new parameters to the model and $\binom{v}{m}$ possible new treatment combinations. With the constraints above, each mixture of level $m$ adds $\sum_{i=0}^{m-1} \binom{Y}{i}$ constraints on the new parameters. The number of new treatment combinations equals the number of new parameters minus the number of constraints on the new parameters. Thus, if treatments involving mixtures of size one up to size $m-1$ are in the design and mixtures of size (level) $m$ are added to the design, the possible number of new treatment combinations is equal to the number of new independent parameters.

Now, remove the restriction that $k \leq \frac{v}{2}$ and consider the extension of the above system of constraints to the case $k > \frac{v}{2}$. $\sum_{h_{k}} \beta_{h_1 h_2 \cdots h_k} = \beta_{h_1 h_2 \cdots h_k}$ is one of the additional constraints. Since there are $\binom{v}{k-1}$ possible combinations of the form $h_1 h_2 \cdots h_{k-1}$, $\binom{v}{k-1}$ constraints are to be placed on the parameters $\beta_{h_1 h_2 \cdots h_k}$. The problem is that if $k > \frac{v}{2}$, then $\binom{v}{k} \leq \binom{v}{k-1}$. This means there are at least as many constraints as there are parameters. This effectively makes all the parameters equal to zero.
The consequence of this is that for \( m > \frac{V}{2} \), the only non-zero new parameters in the model which are not in the model when mixtures of size \( m-1 \) or smaller are considered, are parameters which have a mate in the model where mixtures are of size \( v-m \). The \((m-r)-r\)-specific effect will not be defined as equal to zero if \( r \leq v-m \). The effect \( (m-r)\beta h_1 h_2 \ldots h_r \) will have the same constraints as in cases where \( m \leq \frac{V}{2} \):

\[
\sum_{h_j=1}^{V} (m-r)\beta h_1 h_2 \ldots h_{r-1} h_j = (m-r)\beta h_1 h_2 \ldots h_{r-1} = 0.
\]

If \( r > v-m \), all \((m-r)-r\)-specific effects will be defined as identically zero.

So, the addition of mixtures of size \( m \), to a design already containing mixtures of \((m-1)\) agents, will result in \( \binom{V}{m} \) new independent parameters regardless of whether \( m > \frac{V}{2} \) or \( m \leq \frac{V}{2} \).

For example, with seven possible agents the following table shows the number of parameters and the number of constraints in this general model. Within each cell, the first number tells how many new non-zero parameters of that form there are and the second number tells how many new constraints of that form there are.
Table 0.

<table>
<thead>
<tr>
<th>parameter</th>
<th>number of agents in the mixture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu(n) )</td>
<td>1,0</td>
<td>7,0</td>
</tr>
<tr>
<td>( r_{h_1} )</td>
<td>7,1</td>
<td>7,1</td>
</tr>
<tr>
<td>( (n-\alpha)_{h_1} )</td>
<td>0,0</td>
<td>35, 5</td>
</tr>
<tr>
<td>( \beta_{h_1 h_3} )</td>
<td>0,0</td>
<td>21, 7</td>
</tr>
<tr>
<td>( (r-\beta)_{h_1 h_3} )</td>
<td>0,0</td>
<td>63,21</td>
</tr>
<tr>
<td>( \beta_{h_1 h_3 h_4} )</td>
<td>0,0</td>
<td>35,21</td>
</tr>
<tr>
<td>( (r-\beta)_{h_1 h_3 h_4} )</td>
<td>0,0</td>
<td>35,21</td>
</tr>
<tr>
<td>( \beta_{h_1 h_3 h_4} )</td>
<td>0,0</td>
<td>0,0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8,1</strong></td>
<td><strong>203,76</strong></td>
</tr>
<tr>
<td><strong>Difference</strong></td>
<td>( 7=\binom{7}{1} ) 21=\binom{7}{2} 35=\binom{7}{3} 35=\binom{7}{4} 21=\binom{7}{5} 7=\binom{7}{6} 1=\binom{7}{7} )</td>
<td></td>
</tr>
</tbody>
</table>

In each column total it is to be noted that the difference, parameters minus constraints, is the number of possible combinations of \( n \) agents.

III.3. Estimation

The expected value of the yield of the \( j^{th} \) mixture of \( n \) out of \( v \) agents is:

\[
E(Y_{(n)ij}) = \mu(n) + \sum_{h_1=1}^{v} I_{n,j}(h_1) \left( \frac{1}{n} \left( r_{h_1} + (1-\alpha)_{h_1} + (2-\alpha)_{h_1} + \ldots + ((n-1)-\alpha)_{h_1} \right) \right)
\]

\[
+ \sum_{h_2=h_1+1}^{v} I_{n,j}(h_2) \left( \left( \frac{n}{n} \right)^{-1} \left( \beta_{h_1 h_2} + (1-\beta)_{h_1 h_2} + \ldots + ((n-2)-\beta)_{h_1 h_2} \right) \right)
\]

\[
+ \ldots
\]
In a replicate, \( i \), where all possible combinations of \( n \) agents are present, the parameter \( \mu(n) \) and the \( \binom{n}{v} \) parameters of the form \( \beta h_1 h_2 \ldots h_{n-1} h_n \) are estimable. Also estimable are the linear combinations of parameters which are in brackets above — terms of the form

\[
\left[ \binom{n-1}{k} (\beta h_1 h_2 \ldots h_k + (1-\beta) h_1 h_2 \ldots h_k + \ldots + ((n-k)-\beta) h_1 h_2 \ldots h_k) \right], \quad k = 2, 3, \ldots, n-1.
\]

\[
E\left( \sum_{j=1}^{v} Y(n)_{ij} \right) = \sum_{j=1}^{v} E(Y(n)_{ij})
\]

\[
= \binom{n}{v} \mu(n) + \binom{n-1}{v-1} \sum_{h_1=1}^{n-1} \frac{1}{h_1} (\tau h_1 + (1-\alpha) h_1 + \ldots + ((n-1)-\alpha) h_1) + \ldots
\]

\[
+ \binom{v-2}{h_2=1} \sum_{h_1=1}^{v-2} \binom{n}{2} \left[ \beta h_1 h_2 + (1-\beta) h_1 h_2 + \ldots + ((n-2)-\beta) h_1 h_2 \right] + \ldots
\]

\[
+ \binom{v-1}{h_3=1} \sum_{h_1=1}^{v} \sum_{h_2=1}^{h_1} \binom{n}{h_1} \left[ \beta h_1 h_2 \ldots h_{n-1} + (1-\beta) h_1 h_2 \ldots h_{n-1} \right]
\]

\[
+ \sum_{h_1=1}^{v} \sum_{h_2=1}^{h_1} \sum_{h_3=1}^{h_2} \beta h_1 h_2 h_3 \ldots h_n
\]

With the constraints proposed in the previous section, this reduces to:
Thus $\mu(n)$ is estimated by $\hat{\mu}(n) = \left(\sum_{j=1}^{Y(n)} Y(n)_{ij}\right)^{-1}$.

The expected value of the sum of all mixtures containing agent $a$ is:

$$E\left(\sum_{j=1}^{Y(n)} Y(n)_{ij}\right) = \left(\sum_{j=1}^{Y(n)} Y(n)_{ij}\right)^{-1}$$

\[ E\left(\sum_{j=1}^{Y(n)} I_{nj}(a)Y(n)_{ij}\right) = \sum_{j=1}^{Y(n)} I_{nj}(a)E\left(Y(n)_{ij}\right) \]

\[ = \left(\sum_{j=1}^{Y(n)} Y(n)_{ij}\right)^{-1} \left[\mu(n) + \frac{1}{n} (\tau_a + (1-\alpha)_a + \cdots + ((n-1)-\alpha)_a) \right] \]

\[ + \left(\sum_{j=1}^{Y(n)} \left(\frac{1}{n} (\tau_{h_1} + (1-\alpha)_{h_1} + \cdots + ((n-1)-\alpha)_{h_1}) \right) \right] \]

\[ + \left(\sum_{j=1}^{Y(n)} \left(\frac{1}{n} (\beta_{h_1} + (1-\beta)_{h_1} + \cdots + ((n-2)-\beta)_{h_1} \right) \right] \]

\[ + \left(\sum_{j=1}^{Y(n)} \left(\frac{1}{n} (\beta_{h_1} + (1-\beta)_{h_1} + \cdots + ((n-3)-\beta)_{h_1} \right) \right] \]

\[ + \cdots \]

\[ + \left(\sum_{j=1}^{Y(n)} \left(\frac{1}{n} (\beta_{h_1 h_2} + (1-\beta)_{h_1 h_2} + \cdots + ((n-2)-\beta)_{h_1 h_2} \right) \right] \]

With the constraints proposed in the previous section, this reduces to:

$$E\left(\sum_{j=1}^{Y(n)} I_{nj}(a)Y(n)_{ij}\right) = (n-1)^{\mu(n)} + \left[ (n-1)^{-\frac{1}{n}} - (n-2)^{-\frac{1}{n}} \right] \left(\frac{1}{n} \right) (\tau_a + (1-\alpha)_a + \cdots + ((n-1)-\alpha)_a)$$

\[ = (n-1)^{\mu(n)} + (n-1)^{-\frac{1}{n}} \left(\frac{1}{n} \right) (\tau_a + (1-\alpha)_a + \cdots + ((n-1)-\alpha)_a). \]
The expected value of the sum of all mixtures containing agents $a_1, a_2, \ldots, a_k$ ($n \leq \frac{v}{2}$, $k = 2, 3, \ldots, n$) is:

\[
\mathbb{E}\left( \sum_{j=1}^{n} n_j(a_1)n_j(a_2) \cdots n_j(a_k)Y(n)_{ij} \right) = \sum_{j=1}^{n} n_j(a_1)n_j(a_2) \cdots n_j(a_k)\mathbb{E}(Y(n)_{ij})
\]

\[
= (v-k)\left[ \frac{\mu(n)}{n} + \sum_{\ell=1}^{k} \frac{1}{n} (r_{a_\ell} + (1-\alpha)a_{a_\ell} + (2-\alpha)a_{a_\ell} + \cdots + ((n-1)-\alpha)a_{a_\ell}) \right] \\
+ (v-k-1) \sum_{h=1}^{n-k-1} \frac{1}{n} (r_{a_{a_\ell}} + (1-\alpha)a_{a_{a_\ell}} + (2-\alpha)a_{a_{a_\ell}} + \cdots + ((n-1)-\alpha)a_{a_{a_\ell}}) \\
\text{for } a_{a_\ell} \neq a_{a_\ell} \text{ (} \ell = 1, 2, \ldots, k) \\
+ (v-k) \sum_{\lambda_1=1}^{k} \sum_{\lambda_2=\lambda_1+1}^{k} \frac{(n-1)}{(n-2)} (\beta_{a_{a_{\lambda_1}}a_{a_{\lambda_2}}} + (1-\beta)a_{a_{\lambda_1}}a_{a_{\lambda_2}} + \cdots + (n-2-\beta)a_{a_{\lambda_1}}a_{a_{\lambda_2}}) \\
+ (v-k-1) \sum_{\lambda_1=1}^{k} \sum_{h_1=1}^{n-k} \frac{(n-1)}{(n-2)} (\beta_{a_{a_{\lambda_1}}h_1} + (1-\beta)a_{a_{\lambda_1}}h_1 + \cdots + (n-2-\beta)a_{a_{\lambda_1}}h_1) \\
\text{for } h_1 \neq a_{a_\ell} \text{ (} \ell = 1, 2, \ldots, k)
With the constraints proposed in the previous section, this reduces to:

$$E\left( \sum_{j=1}^{l} I_{nj}(a_1)I_{nj}(a_2) \cdots I_{nj}(a_k)Y(n)_{ij} \right)$$

$$= \binom{v-k}{n-k}E(1) + \binom{v-k-1}{n-k} \sum_{k=1}^{n-k} \binom{n-k}{k} \left[ (1-\alpha)_{\alpha_1} + \cdots + (n-1-\alpha)_{\alpha_2} \right]$$

$$+ \binom{v-k-2}{n-k} \sum_{k=1}^{n-k} \binom{n-k}{k} \left( \beta_{\alpha_1,\alpha_2} + (1-\beta)_{\alpha_1,\alpha_2} + \cdots + (n-2-\beta)_{\alpha_1,\alpha_2} \right)$$

$$+ \cdots + (\binom{v-k-2}{n-k})^{k-1} \left( \beta_{\alpha_1,\alpha_2} \cdots \alpha_k + (1-\beta)_{\alpha_1,\alpha_2} \cdots \alpha_k + \cdots + (n-k-\beta)_{\alpha_1,\alpha_2} \cdots \alpha_k \right).$$

The term $$\left[ (\binom{n}{k})^{-1} \beta_{a_1,\alpha_2} \cdots \alpha_k + (1-\beta)_{a_1,\alpha_2} \cdots \alpha_k + \cdots + (n-k-\beta)_{a_1,\alpha_2} \cdots \alpha_k \right]$$ is estimated by:

$$\left[ (\binom{n}{k})^{-1} \beta_{a_1,\alpha_2} \cdots \alpha_k + (1-\beta)_{a_1,\alpha_2} \cdots \alpha_k + \cdots + (n-k-\beta)_{a_1,\alpha_2} \cdots \alpha_k \right]^*$$

$$= \binom{v-k}{n-k} \left[ \sum_{j=1}^{l} I_{nj}(a_1)I_{nj}(a_2) \cdots I_{nj}(a_k)Y(n)_{ij} \right]$$
By using this recursive formula for estimation, all the bracketed terms in formula (1) can be estimated, as well as \( \mu(n) \) and \( \beta_{h_1 h_2 \cdots h_n} \). This estimation procedure is effective whenever \( n \leq \frac{v}{2} \), the number of agents in a mixture, is not more than half the total number of agents.

With an estimate of \( \left[ \begin{array}{c} n \\ k \end{array} \right] (\beta_{a_1 \cdots a_k} + (1-\alpha)_{a_1} + \cdots + (n-1-\alpha)_{a_k}) \), \( \left[ \begin{array}{c} n \\ k \end{array} \right] \) times that provides an estimate of \( (\beta_{a_1 \cdots a_k} + (1-\alpha)_{a_1} + \cdots + \left( (n-k-\beta)_{a_1 \cdots a_k} \right) \) when \( n \), the number of agents in a mixture, is allowed to vary, the terms \( \beta_{a_1 \cdots a_k} + (1-\alpha)_{a_1} + \cdots + \left( (n-k-\beta)_{a_1 \cdots a_k} \right) \) may be partitioned into estimable parts. Consider \( n' < n \leq \frac{v}{2} \) \( k = 2, 3, \cdots, n \), where \( n' \) and \( n \) are sizes of mixtures which have been observed. The procedure described above provides estimates.
\[
\beta_{(kn')} = (\beta_{a_1 \ldots a_k} + (1-\beta)_{a_1 \ldots a_k} + \cdots + (n'-k-\beta)_{a_1 \ldots a_k})^*
\]

and

\[
\beta_{(kn)} = (\beta_{a_1 \ldots a_k} + (1-\beta)_{a_1 \ldots a_k} + \cdots + (n-k-\beta)_{a_1 \ldots a_k})^*
\]

The difference \(\beta_{(kn)} - \beta_{(kn')}\) estimates

\[
((n'-k+1-\beta)_{a_1 \ldots a_k} + (n'-k+2-\beta)_{a_1 \ldots a_k} + \cdots + (n-k-\beta)_{a_1 \ldots a_k}).
\]

If all possible mixtures of less than or equal to \(n\) agents are observed, the components of the term \(\beta_{(kn)}\) may all be estimated by this difference method.

When \(n > \frac{v}{2}\), if \(k\) is allowed to vary from 2 to \(v-n\) instead of to \(n\), formulas (1) to (7) are appropriate. Thus, if \((n-m-\beta)_{\h_1 \ldots \h_a}\) is not defined as equaling zero, it may be estimated using the recursive formula (8).
IV. Simplifying the Model

For the specific experimental situation a general design will contain parameters which are known to have zero value, parameters which the experimenter is willing to assume have zero value, or linear combinations of parameters which should be considered as one parameter.

The examples from chemistry and genetics provide examples of situations where the general model is needlessly complicated. In both cases, all mixtures being compared must contain the same number of agents. In the general model equation,

\[ Y_{(n)ij} = \mu(n) + \sum_{h_1=1}^{v} I_{nj}(h_1) \left( \frac{1}{n} [\tau_{h_1} + (1-\alpha)_{h_1} + (2-\alpha)_{h_1} + \cdots + (n-1-\alpha)_{h_1}] \right) \]

\[ + \sum_{h_3=h_1+1}^{v} I_{nj}(h_3) \left( \left( \frac{n-1}{2} \right) [\beta_{h_1 h_3} + (1-\beta)_{h_1 h_3} + \cdots + (n-2-\beta)_{h_1 h_3}] \right) \]

\[ + \cdots + \sum_{h_n=h_{n-1}+1}^{v} I_{nj}(h_n) (\beta_{h_1 h_2 \cdots h_n}) \cdots ) + \rho_i + \epsilon(n)_{ij}, \]

each of the bracketed ([ ] ) terms is a linear combination of parameters, which is estimable. In the case where only one size mixture is under consideration, these bracketed terms cannot be partitioned into estimable parts. Thus, each bracketed term may be given a single denotation and treated as one parameter. Since comparable parameters all have the same coefficients in these examples, another simplification would be to have one as the only positive coefficient. The term \[ \left[ \tau_{h_1} + (1-\alpha)_{h_1} + \cdots + (n-1-\alpha)_{h_1} \right] \] will be denoted \( \alpha_{h_1} \). This corresponds to the general combining ability of agent (parent) \( h_1 \) in the genetics example. For \( m = 2, 3, \cdots, n \), the term \[ \left[ \beta_{h_1 h_2 \cdots h_n} + (1-\beta)_{h_1 \cdots h_n} + \cdots + (n-m-\beta)_{h_1 \cdots h_n} \right] \] will
be denoted \( \gamma_{h_1 h_2 \cdots h_n} \). This is an \( m \)-specific mixing effect. The model equation is now:

\[
Y(n)_{ij} = \mu(n) + \rho_i + \epsilon(n)_{ij} + \sum_{h_1=1}^{v} I_{n_j}(h_1)(\alpha_{h_1} + \sum_{h_2=h_1+1}^{v} I_{n_j}(h_2)(\gamma_{h_1 h_2} + \sum_{h_3=h_2+1}^{v} I_{n_j}(h_3)(\gamma_{h_1 h_2 h_3} + \cdots + \sum_{h_n=h_{n-1}+1}^{v} I_{n_j}(h_n)(\gamma_{h_1 h_2 \cdots h_n} \cdots)) + \sum_{h_n=h_{n-1}+1}^{v} I_{n_j}(h_n)(\gamma_{h_1 h_2 \cdots h_n} \cdots)) + \epsilon(n)_{ij} \tag{9}
\]

This model equation is appropriate whenever the number of agents in a mixture is not allowed to vary.

The same simplifying notation may be used when \( m \)-specific effect depends only on whether there are at least \( m \) agents in the mixture. The experimenter may be assuming the effects to act this way or know that they do. The claim is that

\[
\left[ \beta_{h_1} \cdots h_n + (1-\beta)_{h_1} \cdots h_n + \cdots + \left( (n-m)-\beta \right)_{h_1} \cdots h_n \right]
\]

is invariant for all \( n \geq m \). The term used to denote this will be \( \gamma_{h_1 \cdots h_n} \). It may still be reasonable to consider a "pure" effect separate from the general mixing effect by denoting \( \left[ (1-\alpha)_{h_1} + (2-\alpha)_{h_1} + \cdots + (n-1)-\alpha \right]_{h_1} \) as \( \alpha'_{h_1} \), invariant for all \( n \geq 2 \). The model equation is now:

\[
Y(n)_{ij} = \mu(n) + \rho_i + \sum_{h_1=1}^{v} I_{n_j}(h_1)(r_{h_1} + \alpha'_{h_1} + \sum_{h_2=h_1+1}^{v} I_{n_j}(h_2)(\gamma_{h_1 h_2} + \cdots + \sum_{h_n=h_{n-1}+1}^{v} I_{n_j}(h_n)(\gamma_{h_1 h_2 \cdots h_n} \cdots)) + \epsilon(n)_{ij} \tag{10}
\]
As in factorial designs, higher order effects can sometimes be eliminated from the model equation. This may be done because of prior knowledge of the behavior of the material under observation. It may be due to simplifying assumptions the experimenter makes about his material. If it is decided that there are no effects of higher order than the m-specific effects, the general model equation becomes:

\[ Y(n)_{ij} = \mu(n) + \rho_i + \epsilon(n)_{ij} + \sum_{h_1=1}^{V} I_{nj}(h_1) \left( \frac{1}{n} \tau_{h_1} + (1-\alpha)_{h_1} + \cdots + ((n-1)-\alpha)_{h_1} \right) \]

\[ + \sum_{h_2=h_1+1}^{V} I_{nj}(h_2) \left( \left( \frac{n}{2} \right)^{-1} \beta_{h_1 h_2} + (1-\beta)_{h_1 h_2} + \cdots + ((n-2)-\beta)_{h_1 h_2} \right) + \cdots \]

\[ + \sum_{h_s=h_{s-1}+1}^{V} I_{nj}(h_m) \left( \left( \frac{n}{m} \right)^{-1} \beta_{h_1 \cdots h_s} + (1-\beta)_{h_1 \cdots h_s} + \cdots + ((n-m)-\beta)_{h_1 \cdots h_s} \right) \cdots \] .

The first simplification (9) becomes:

\[ Y(n)_{ij} = \mu(n) + \rho_i + \epsilon(n)_{ij} + \sum_{h_1=1}^{V} I_{nj}(h_1) \alpha_{h_1} + \sum_{h_2=h_1+1}^{V} I_{nj}(h_2) \gamma_{h_2 h_2} + \cdots \]

\[ + \sum_{h_s=h_{s-1}+1}^{V} I_{nj}(h_m) \left( \gamma_{h_2 \cdots h_s} \cdots \right) . \]

The second simplification (10) is the same as (9) with \( \alpha_{h_1} \) replaced by \( \tau_{h_1} + \alpha'_{h_1} \).
Another possible simplification would be the elimination of $\rho_1$ from the model equation. This could be done whenever the experimenter has only one replicate or the design is a completely randomized design, with no replicates to block on.

A change in the model which would change the constraints on remaining parameters as well as eliminating some parameters would be needed if the application of only one agent was best interpreted as a "mixture" involving two identical agents. "Pure" strains would then belong in the set of mixtures of two agents. The term $\tau_{h_1}$ no longer has any meaning in this case. The model equation for a "mixture" of the first agent is:

$$y(2)_{11} = \mu(2) + \rho_1 + \alpha_1 + \epsilon(2)_{11} + \beta_{11}.$$  

The model equation for a mixture of the first two agents is:

$$y(2)_{12} = \mu(2) + \rho_1 + \frac{1}{2}\alpha_1 + \frac{1}{2}\alpha_2 + \beta_{12} + \epsilon(2)_{12}.$$  

The new parameter is the $\beta_{jj}$ and it necessitates a change in constraints. Where the constraint was

$$\sum_{h_{2}=1}^{v} \beta_{h_{1}h_{2}} = 0 \quad \text{for all } h_{1} = 1, 2, \ldots, v,$$

it becomes

$$\sum_{h_{2}=1}^{v} \beta_{h_{1}h_{2}} = 0 \quad \text{for all } h_{1} = 1, 2, \ldots, v.$$  

The first simplification proposed here was only a simplification of notation. One result of all the other simplifications is a decrease in the number of independent parameters in the model. This has been done either by eliminating parameters from the model or by making one parameter out of many. With fewer par-
ameters it may be possible to estimate all the elements of the model with replicates which do not involve all the possible combinations of agents. A minimal design, a fractional replicate containing as few observations as possible, would be a worthwhile consideration whenever cost or time limited experimental options.
V. Minimal Designs

V.I. Simplest Analog to the Genetics Model

The diallel cross design from genetics has the model equation:

$$Y_{hij} = \mu + \rho_h + \alpha_i + \alpha_j + \beta_{ij} + \epsilon_{hij}$$

where $\alpha_i$ and $\alpha_j$ represent general combining abilities and $\beta_{ij}$ represents bi-specific combining ability. A simplified form of the general mixing design generalizes this design to the situation when the number of agents in a mixture is not necessarily two:

$$Y_{(n)ij} = \mu(n) + \rho_i + \sum_{h_1=1}^{v} I_{n,j}(h_1)(k(n)\alpha_{h_1}) + \sum_{h_2=h_1+1}^{v} I_{n,j}(h_2)k'(n)\beta_{n_1}n_2$$

$$+ \epsilon(n)_{ij} . \quad (11)$$

When only mixtures all with the same number of agents are being considered, $k(n)$ and $k'(n)$ will both equal one. When different size mixtures are of interest, $k(n)$ and $k'(n)$ are constant coefficients, dependent on the number of agents in the mixture, which adjust the amount of the effect for the amount of the agent which is applied. For $1 < n < v$, this model equation may be applicable.

The mixture is one unit to which many agents have been applied. The block is one conceptual unit to which many treatments have been applied. The mixtures of interest each involve some subset of the set of all possible agents. A block to which not all possible treatments have been applied is called an incomplete block. Thus, in order to make use of the available theoretical work, the mixtures under consideration can be viewed as incomplete blocks, for which only the block totals can be observed. The goal is, then, an incomplete block design which allows esti-
mation of all the parameters in the model with as few observations as possible. This will be called a minimal design.

The goal here is to find minimal designs for estimating general mixing effects (gme's, $\alpha_{h1}'s$) and bi-specific mixing effects (bme's, $\beta_{h1h2}'s$) for fixed number of agents per mixture. Each replicate will be complete, containing all combinations of mixtures which appear anywhere in the design. Without loss of generality, for this complete block (replicate) design, a single replicate may be used when considering estimability of general and bi-specific mixing effects. The yield equation (11) effectively becomes:

$$y(n)_j = \mu(n) + \sum_{h_1=1}^{v-1} \sum_{h_2=h_1+1}^{v} \sum_{j=1}^{n_j} \left( \alpha_{h_1} + \beta_{h_1h_2} \right) + \epsilon(n)_j. \quad (12)$$

For the model equation (11), the degrees of freedom when rs objects are observed — r replicates and s objects per replicate — may be partitioned as in Table 1.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ($\mu(n)$)</td>
<td>1</td>
</tr>
<tr>
<td>General mixing effect ($\alpha_{h_1}$)</td>
<td>v-1</td>
</tr>
<tr>
<td>Bi-specific mixing effect ($\beta_{h_1h_2}$)</td>
<td>$\binom{v(v-3)}{2}$</td>
</tr>
<tr>
<td>Replicates</td>
<td>r-1</td>
</tr>
<tr>
<td>Residual within replicates</td>
<td>$(s-\binom{v}{2})$</td>
</tr>
<tr>
<td>Error</td>
<td>$(r-1)(s-1)$</td>
</tr>
<tr>
<td>Total</td>
<td>rs</td>
</tr>
</tbody>
</table>
The residual within replicates is the amount of variation which would be accounted for by considering higher order specific mixing effects after eliminating general and bi-specific mixing effects. If these higher order effects are zero, this line provides a second estimate of error.

For model equation (12), the degrees of freedom when s objects are observed and all parameters are estimable may be partitioned as in Table 2.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (μ(n))</td>
<td>1</td>
</tr>
<tr>
<td>General mixing effect (αh1)</td>
<td>v-1</td>
</tr>
<tr>
<td>Bi-specific mixing effect (αh1h2)</td>
<td>(\frac{1}{2}v(v-3))</td>
</tr>
<tr>
<td>Residual</td>
<td>s - (\frac{1}{2}v(v-1))</td>
</tr>
<tr>
<td>Total</td>
<td>s</td>
</tr>
</tbody>
</table>

In this model the contribution of residual is to provide a confidence interval for the estimates of effects. The "error" from Table 1 provides a better confidence interval for the estimates. The minimal design will, ideally, leave zero degrees of freedom for residual.

The yield equations of form (12) can be put in matrix form:

\[
\begin{bmatrix}
1 \\
A \\
N
\end{bmatrix}
\begin{bmatrix}
\mu \\
α \\
θ
\end{bmatrix}
+ \mathbf{e} = \mathbf{Y}
\]

where \(1\) is \((s \times 1)\), \(A\) is \((s \times v)\), \(N\) is \((s \times \binom{v}{2})\), \(α\) is \((v \times 1)\), and \(θ\) is \((\binom{v}{2} \times 1)\).
\[ \alpha' = (\alpha_1 \alpha_2 \cdots \alpha_v), \quad \beta' = (\beta_{12} \beta_{13} \cdots \beta_{1v} \beta_{23} \cdots \beta_{2v} \cdots \beta_{(v-1)v}), \quad \text{and} \]
\[ Y' = (Y(n)b_1 \ Y(n)b_2 \cdots \ Y(n)b_s). \]

Let \( a_m' \) be the \( m \text{th} \) row of \( A \) and \( n_m' \) be the \( m \text{th} \) row of \( N \).
\[ a_m' = (I_{nb_a}(1) \ I_{nb_a}(2) \ I_{nb_a}(3) \cdots \ I_{nb_a}(v)). \]
\[ n_m' = (I_{nb_a}(1) \cdot I_{nb_a}(2) \ I_{nb_a}(1) \cdot I_{nb_a}(3) \cdots \ I_{nb_a}(v) \cdot I_{nb_a}(2) \cdot I_{nb_a}(3) \cdots \ I_{nb_a}(2) \cdot I_{nb_a}(v) \cdots I_{nb_a}(v-1) \cdot I_{nb_a}(v)). \]
\[ e' = (e(n)b_1 \ e(n)b_2 \cdots \ e(n)b_s). \]

**Theorem 1:** It is a sufficient condition for the estimability of parameters in the model equation
\[ Y(n)_j = \mu(n) + \sum_{h_1=1}^{v} I_{n_j}(h_1)(\alpha_{h_1} + \sum_{h_2=h_1+1}^{v} I_{n_j}(h_2)\beta_{h_1 h_2}) + e(n)_j \]
with constraints
\[ \sum_{h_1=1}^{v} \alpha_{h_1} = \sum_{i=1}^{v} \beta_{i j} = 0 \quad \text{(for all } j = 1, 2, \cdots, v) \]
that \( N \) in
\[ Y = [1 \ A \ N] \begin{bmatrix} \mu \\ \vdots \\ \alpha \\ \beta \end{bmatrix} + e \]
be of full column rank.
Proof: Let

$$\hat{\beta}_{h_1 h_2} = \binom{n}{2}^{-1} \mu(n) + \binom{n-1}{1}^{-1} (\alpha_{h_1} + \alpha_{h_2}) + \beta_{h_1 h_2}.$$ 

The yield equations

$$\mathbf{y} = \left[ \begin{array}{c} \mu \\ \alpha \\ \mathbf{e} \end{array} \right] + \mathbf{e} \text{ become } \mathbf{y} = [N] \left[ \hat{\beta}^I \right] + \mathbf{e}. $$

$$\hat{\beta}^I = \begin{pmatrix} \beta_{12}^I & \beta_{13}^I & \cdots & \beta_{1v}^I & \beta_{23}^I & \cdots & \beta_{2v}^I & \cdots & \beta_{(v-1)v}^I \end{pmatrix} .$$

If \( N \) has full column rank, \( N'N \) is invertible. By simple matrix multiplication, \( \hat{\beta}^I \) is estimated by \( \hat{\beta}^I = (N'N)^{-1} N'y. \)

$$\hat{\beta}_{h_1 h_2} = \left( \binom{n}{2}^{-1} \mu(n) + \frac{1}{n-1} (\alpha_{h_1} + \alpha_{h_2}) \right).$$

This may be considered as a linear combination of estimates, \( \mu(n) \), \( \alpha_{h_1} \), \( \alpha_{h_2} \), and \( \beta_{h_1 h_2} \), of the parameters, \( \mu(n) \), \( \alpha_{h_1} \), \( \alpha_{h_2} \), and \( \beta_{h_1 h_2} \). The same constraints hold on these estimates,

$$\sum_{h_1=1}^{v} \sum_{h_2=h_1+1}^{m-1} c_{h_1 h_2}^* = \sum_{h_1=1}^{v} \sum_{h_2=h_1+1}^{v} \beta_{h_1 h_2}^* = 0.$$

$$\hat{\beta}^I = \sum_{h_1=1}^{v} \sum_{h_2=h_1+1}^{v} \hat{\beta}_{h_1 h_2}^* = \sum_{h_1=1}^{v} \sum_{h_2=h_1+1}^{v} \left[ \binom{n}{2}^{-1} \mu(n) + (n-1)^{-1} (\alpha_{h_1} + \alpha_{h_2}) + \beta_{h_1 h_2}^* \right]$$

$$= \left( \binom{v}{2} \right)^{-1} \mu(n) + \sum_{h_1=1}^{v} (n-1)(n-1)^{-1} (\alpha_{h_1}^*) + \sum_{h_1=1}^{v} \sum_{h_2=h_1+1}^{v} \beta_{h_1 h_2}^*$$

$$= \left( \binom{v}{2} \right)^{-1} \mu(n). \text{ So } \mu(n) = \left( \binom{v}{2} \right)^{-1} \hat{\beta}^I.$$
\[
\sum_{h_0=2}^{v} \hat{\beta}_{1h_0}^I = \sum_{h_0=2}^{v} \left[ \frac{(n)}{2} \mu_{(n)} + (n-1)^{-1}(\alpha_1^* + \alpha_{h_0}^*) + \beta_{1h_0}^\mu \right]
\]

\[
= (v-1)\left(\frac{n}{2}\right) \mu_{(n)} + (v-1)(n-1)^{-1} \alpha_1^* + \sum_{h_0=2}^{v} \alpha_{h_0}^* + \sum_{h_0=2}^{v} \beta_{1h_0}^I
\]

\[
= (v-1)\left(\frac{n}{2}\right) \mu_{(n)} + (v-1)(n-1) - 1 \alpha_1^*. \text{ So } \alpha_1^* = \left(\frac{n-1}{v-n}\right) \sum_{h_0=2}^{v} \hat{\beta}_{1h_0}^I - (v-1)\left(\frac{v}{2}\right)^{-1} \hat{\beta}_1^I
\]

Similarly:

\[
\alpha_2^* = \left(\frac{n-1}{v-n}\right) \left[ \beta_{12}^I + \sum_{h_0=3}^{v} \hat{\beta}_{2h_0}^I - (\frac{2}{v})^I \hat{\beta}_2^I \right],
\]

\[
\alpha_3^* = \left(\frac{n-1}{v-n}\right) \left[ \sum_{h_1=1}^{2} \hat{\beta}_{h_13}^I + \sum_{h_0=4}^{v} \hat{\beta}_{3h_0}^I - (\frac{2}{v})^I \hat{\beta}_3^I \right],
\]

\[\vdots\]

\[
\alpha_v^* = \left(\frac{n-1}{v-n}\right) \left[ \sum_{h_1=1}^{v-1} \hat{\beta}_{h_1v}^I - (\frac{2}{v})^I \hat{\beta}_v^I \right].
\]

\[
\hat{\beta}_{h_1h_0}^I = \left(\frac{n}{2}\right)^{-1} \mu_{(n)} + (n-1)^{-1}(\alpha_{h_1}^* + \alpha_{h_0}^*) + \beta_{h_1h_0}^\mu.
\]

So

\[
\beta_{h_1h_0}^* = \beta_{h_1h_0}^I - (v-1)\left(\frac{v}{v-n}\right) \sum_{i=1}^{h_1-1} \hat{\beta}_{ih_1}^I + \sum_{i=h_1+1}^{v} \hat{\beta}_{i1h_1}^I + \sum_{j=1}^{h_0-1} \hat{\beta}_{j1h_0}^I + \sum_{j=h_0+1}^{v} \hat{\beta}_{j1h_0}^I - (\frac{2}{v})^I \hat{\beta}_1^I.
\]

Thus, all the parameters may be estimated.
A minimal design for which $N$ has full column rank has the same number of rows as columns. With $v$ possible agents, a full-column rank

$$N((\frac{v}{2}) \times (\frac{v}{2}))$$

may be found whenever $2 \leq n \leq (v-2)$. Though the goal is estimation of parameters with a minimum number of observations, it may be possible to retain some favorable qualities of the design which considers all possible combinations of agents. Equal application of each agent and equal application of each pair of agents are design characteristics which may be retained in some circumstances. When agents are considered as treatments and objects are considered as blocks, these design characteristics indicate a balanced incomplete block design (BIB).

**Definition 1:** A BIB design is **irreducible** if its blocks cannot be split into two mutually exclusive subsets, both of which are BIB designs.

**Theorem 2:** Assume that for blocks of size $n$, with $v$ treatments, there exists a BIB design containing $\left( \binom{v}{2} \right)$ blocks. For such a design to be minimal it must be irreducible.

**Proof:** Let a BIB design exist which is minimal and produces the yield equations

$$Y = [N]\beta^I + \varepsilon.$$ Assume there exists a reordering of $Y$, call it $\left[ \frac{Y_1}{Y_2} \right]$, and a similar reordering of the rows of $N$, $\left[ -\frac{N_1}{N_2} \right]$, and of $\varepsilon$, $\left[ -\frac{\varepsilon_1}{\varepsilon_2} \right]$, such that

$$Y_1 = N_1\beta^I + \varepsilon_1$$

represents the yield equations for a BIB design. The sum of the rows of $N_1$ will be $\lambda_1'1'$ where $\lambda_1$ is the number of times each pair of agents occurs in the design. Similarly, the sum of the rows of $N$ will be $\lambda_1'1'$ where $\lambda$ is the number of times each pair of agents occurs in the original design. This means the sum of the rows of $N_2$ must be $(\lambda - \lambda_1)1'$. The augmented matrix $\left( -\frac{N_1}{N_2} \right)$ can have rank at most $\left( \binom{v}{2} \right) - 1$. Rank of $\left( -\frac{N_1}{N_2} \right) = \text{Rank of } N$ so $N$ is not of full rank. This is a contradiction of the minimality of the BIB design, so the minimal BIB design must be irreducible.
For \( n = 2 \) or \( n = (v-2) \), the set of all possible combinations of agents is a minimal design. Table 3 is a partial list of the values for \( v \) and \( n \) for which a minimal BIB design may exist. A general method for the construction of minimal BIB designs has not been discovered. Starred entries in the table are values for which a minimal BIB design has been found.

<table>
<thead>
<tr>
<th>Treatments ((v))</th>
<th>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments/block ((n))</td>
<td>3 4 4 3 4 5 6 4 5 6 3 4 5 6 7 8 4 6 8 5 8</td>
</tr>
</tbody>
</table>

If there are \( v \) treatments and a block \( b \) contains \( n \) of the treatments, a block \( b' \) containing the \((v-n)\) treatments not in \( b \) is called the complement of block \( b \). It is easily verified that if the set \( b_1, b_2, \ldots, b_m \) forms a BIB design, then the set \( b'_1, b'_2, \ldots, b'_m \) forms a BIB design.

**Theorem 3:** If a minimal BIB design exists for \( v \) treatments and \( n \) treatments per block, then a minimal BIB design exists for \( v \) treatments and \((v-n)\) treatments per block.

**Proof:** Let the set of blocks \( b_1, b_2, \ldots, b_v \) be a minimal BIB design for \( n \) treatments per block. The set of complements \( b'_1, b'_2, \ldots, b'_v \) must be a BIB. Since the set \( b_1, b_2, \ldots, b_v \) forms an irreducible BIB, no subset is a BIB. This implies no subset of \( b'_1, b'_2, \ldots, b'_v \) can form a BIB. Thus \( b'_1, b'_2, \ldots, b'_v \) is an irreducible BIB with \((v-n)\) treatments per block and \( \binom{v}{2} \) blocks. This is a minimal BIB.
V.2. Generalizing With More Specific Mixing Effects

When only mixtures of one size are to be considered and the model is to include not only bi-specific but also tri-specific up to r-specific mixing effects, the simplified yield equation comparable to equation (12) is:

\[ Y(n)_j = \mu(n) + \sum_{h_1=1}^{v} I_{nj}(h_1)(\alpha_{h_1}) + \sum_{h_2=h_1+1}^{v} I_{nj}(h_2)(\beta_{h_1 h_2}) + \ldots \]

\[ + \sum_{h_r=h_{r-1}+1}^{v} I_{nj}(h_r)(\beta_{h_1 h_2 \ldots h_r}) \ldots ) + \epsilon(n)_j. \quad (13) \]

The constraints require that \( r \leq \frac{V}{2} \), or the model could be simplified by eliminating the m-specific effects for all \( m > \frac{V}{2} \). For all parameters to be estimable, there must be at least as many possible observations as there are independent parameters. This is taken care of by the restriction on \( n \): \( r \leq n \leq v-r \). The yield equations can be written as:

\[
Y = \begin{bmatrix}
1 & A & N_2 & N_3 & \ldots & N_r
\end{bmatrix}
\begin{bmatrix}
\mu(n) \\
\alpha \\
\beta_2 \\
\beta_3 \\
\vdots \\
\beta_r
\end{bmatrix} + \epsilon.
\]

\( \beta_k \) is the vector of the \( \binom{v}{k} \) different k-specific mixing effects.

Theorem 4: A sufficiency condition for estimability, similar to that for equation (12), is that \( N_r \) has full column rank.
Proof: Just as in the proof of Theorem 1, it is possible to identify a parameter

$$\beta_{h_1 \ldots h_r} = (n^{-1}) \mu(n) + (n-1)^{-1} \sum_{i=1}^{r} \beta_{h_i} + \cdots + (n-r+1)^{-1} (\beta_{h_{r-1}} + \beta_{h_{r-2}h_r} + \cdots + \beta_{h_{r-1} \ldots h_r}) + \beta_{h_1 \ldots h_r}.$$ 

Let $N_r$ have full column rank. The yield equation may be written as $Y = N_r \beta^{II} + \epsilon$. $N_r^t N_r$ is invertible, so $\hat{\beta}^{II} = (N_r^t N_r)^{-1} N_r^t Y$ is an estimate of $\beta^{II}$. $\hat{\beta}_{h_1 \ldots h_r}$ has a form similar to the form of model equation (10) without the error term. The vector $\hat{\beta}^{II}$ contains an estimate of the effect $\beta_{h_1 \ldots h_r}$ for all $\left(\begin{array}{c} m \\end{array}\right)$ possible combinations of $r$ treatments. Using the method of estimation described in section III.3, all the parameters which make up $\beta_{h_1 \ldots h_r}$ can be solved for. Thus, all the parameters in the original yield equation are estimable.

In the simple case it was advantageous to have all pairs of agents occur the same number of times. The generalization of this would have all sets of $r$ agents occur the same number of times. A minimal design has $\left(\begin{array}{c} m \\end{array}\right)$ different objects being treated. BIB designs require pairwise balance. Let $m$-wise balance mean that all sets of $m$ agents occur the same number of times; $m$-wise balance implies $(m-1)$-wise balance. Just as in the simple case it was not always possible to have a pairwise balanced minimal design, in the general case it is not always possible to have an $m$-wise balanced minimal design ($m = 2, 3, \ldots, r$). The treatment design to seek, for fixed $v, n, r$, is one which maximizes $m$, where $m$-wise balance is achieved by the design and $(m+1)$-wise is not.

Trio-wise balanced incomplete block designs have been discussed by Calvin (1954), calling them "doubly balanced incomplete block designs". Though the names are similar, the $n$-wise balanced design spoken of here is unrelated to the balanced $n$-ary design introduced by Tocher (1952).
VI. An Example

An experimenter has seven different agents and plans to apply three of them to each experimental unit. The first model to be considered has yield equation (13) with \( r = 3, n = 3 \).

\[
y_{(3)j} = \mu_{(3)} + \sum_{h_1=1}^{7} I_{3j}(h_1)(\alpha_h) + \sum_{h_2=h_1+1}^{7} I_{3j}(h_2)(\beta_{h_1h_2}) + \sum_{h_3=h_2+1}^{7} I_{3j}(h_3)(\beta_{h_1h_2h_3}) + \epsilon(n)_j.
\]

(14)

All possible combinations of three agents are denoted \( D_{35} \):

\[
\begin{array}{cccccccccccccccc}
\hline
D_{35} & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\
& 2 & 2 & 2 & 2 & 2 & 2 & 4 & 4 & 4 & 5 & 5 & 5 & 5 & 5 & 5 & 5 & 5 & 5 \\
\hline
\end{array}
\]

With the constraints

\[
\sum_{h_1=1}^{7} \alpha_{h_1} = \sum_{h_1=1}^{7} \beta_{h_1h_2} = \sum_{h_1=1}^{7} \beta_{h_1h_2h_3} = 0,
\]

all the parameters are easily estimated.
\[
E\left( \sum_{j=1}^{35} y_{(3)j} \right) = 35 \mu_{(3)} + 15 \sum_{h_1=1}^{7} \alpha_{h_1} + 5 \sum_{h_1=1}^{6} \sum_{h_2=h_1+1}^{7} \beta_{h_1 h_2} + \sum_{h_1=1}^{5} \sum_{h_2=h_1+1}^{6} \sum_{h_3=h_2+1}^{7} \beta_{h_1 h_2 h_3}
\]

\[
= 35 \mu_{(3)} \quad \text{So } \mu_{(3)}^2 = \frac{1}{35} \sum_{j=1}^{35} y_{(3)j}.
\]

\[
E\left( \sum_{j=1}^{35} I_{3j}^{(k)} y_{(3)j} \right) = 15 \mu_{(3)} + 10 \alpha_{k} + 5 \sum_{h_1=1}^{7} \alpha_{h_1} + 4 \left( \sum_{h_1=1}^{k-1} \beta_{h_1 k} + \sum_{h_2=k+1}^{7} \beta_{kh_2} \right)
\]

\[
+ \sum_{h_1=1}^{6} \sum_{h_2=h_1+1}^{7} \beta_{h_1 h_2} + \sum_{h_1=1}^{k-1} \sum_{h_2=h_1+1}^{7} \beta_{h_1 h_2} + \sum_{h_1=1}^{6} \sum_{h_2=h_1+1}^{7} \beta_{h_1 h_2 h_3}
\]

\[
= 15 \mu_{(3)} + 10 \alpha_{k} \quad \text{So } \alpha_{k}^2 = \frac{1}{10} \left( \sum_{j=1}^{35} I_{3j}^{(k)} y_{(3)j} - \frac{15}{35} \sum_{j=1}^{35} y_{(3)j} \right).
\]

\[
E\left( \sum_{j=1}^{35} I_{3j}^{(k_1)} I_{3j}^{(k_2)} y_{(3)j} \right) = 5 \mu_{(3)} + 4 \alpha_{k_1} + 4 \alpha_{k_2} + \sum_{h_1=1}^{7} \alpha_{h_1} + 3 \beta_{k_1 k_2}
\]

\[
+ \sum_{h_1=1}^{k_1-1} \beta_{h_1 k_1} + \sum_{h_3=k_1+1}^{7} \beta_{k_1 h_3} + \sum_{h_1=1}^{k_2-1} \beta_{h_1 k_2} + \sum_{h_3=k_1+1}^{7} \beta_{k_2 h_3}
\]

\[
+ \sum_{h_1=1}^{k_1-1} \sum_{h_2=k_1+1}^{k_2-1} \beta_{h_1 k_1 k_2} + \sum_{h_1=1}^{k_2-1} \sum_{h_3=k_1+1}^{k_3-1} \beta_{k_1 h_2 k_3} + \sum_{h_3=k_1+1}^{7} \beta_{k_1 k_2 h_3}
\]

\[
= 5 \mu_{(3)} + 4 \alpha_{k_1} + 4 \alpha_{k_2} + 3 \beta_{k_1 k_2}.
\]
\[
\beta_{k_1 k_2} = \frac{1}{3} \left( \sum_{j=1}^{35} I_{3j}(k_1)I_{3j}(k_2)Y_{(3)j} \right) - \frac{4}{10} \left( I_{3j}(k_1)Y_{(3)j} + I_{3j}(k_2)Y_{(3)j} - \frac{30}{35} Y_{(3)j} \right)
- \frac{5}{35} Y_{(3)j} \right)
= \frac{1}{3} \left( \sum_{j=1}^{35} I_{3j}(k_1)I_{3j}(k_2)Y_{(3)j} \right) - \frac{4}{10} \left( I_{3j}(k_1)Y_{(3)j} + I_{3j}(k_2)Y_{(3)j} \right) + \frac{1}{5} Y_{(3)j} \right).
\]

\[
E \left( \sum_{j=1}^{35} I_{3j}(k_1)I_{3j}(k_2)I_{3j}(k_3)Y_{(3)j} \right) = \mu_{(3)} + \alpha_{k_1} + \alpha_{k_2} + \alpha_{k_3} + \beta_{k_1 k_2} + \beta_{k_1 k_3} + \beta_{k_2 k_3}.
\]

\[
\beta_{k_1 k_2 k_3} = \sum_{j=1}^{35} I_{3j}(k_1)I_{3j}(k_2)I_{3j}(k_3)Y_{(3)j}
- \frac{1}{3} \sum_{j=1}^{35} \left[ I_{3j}(k_1)I_{3j}(k_2)Y_{(3)j} + I_{3j}(k_1)I_{3j}(k_3)Y_{(3)j} + I_{3j}(k_2)I_{3j}(k_3)Y_{(3)j} \right]
+ \frac{1}{6} \sum_{j=1}^{35} \left[ I_{3j}(k_1)Y_{(3)j} + I_{3j}(k_2)Y_{(3)j} + I_{3j}(k_3)Y_{(3)j} \right] - \frac{1}{10} \sum_{j=1}^{35} Y_{(3)j}.
\]

Table 4 is the analysis of variance table for this design with sums of squares expressed in terms of the estimates \(\beta_{(3)}, \alpha_{h_1}, \beta_{h_1 h_2}, \text{ and } \beta_{h_1 h_2 h_3}\) as formulated above.
Table 4. Analysis of Variance

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ( \mu_{(3)} )</td>
<td>1</td>
<td>( 35\mu_{(3)}^2 )</td>
</tr>
<tr>
<td>General Mixing Effect ( (\alpha_{h_1}) )</td>
<td>6</td>
<td>( \sum_{h_1=1}^{5} \sum_{h_2=h_1+1}^{6} \sum_{h_3=h_2+1}^{2} (\alpha_{h_1}^* + \alpha_{h_2}^* + \alpha_{h_3}^*)^2 )</td>
</tr>
<tr>
<td>Bi-specific Mixing Effect ( (\beta_{h_1 h_2}) )</td>
<td>14</td>
<td>( \sum_{h_1=1}^{5} \sum_{h_3=h_1+1}^{6} \sum_{h_3=h_2+1}^{7} (\beta_{h_1 h_3}^* + \beta_{h_1 h_3}^* + \beta_{h_2 h_3}^*)^2 )</td>
</tr>
<tr>
<td>Tri-specific Mixing Effect ( (\beta_{h_1 h_2 h_3}) )</td>
<td>14</td>
<td>( \sum_{h_1=1}^{5} \sum_{h_2=h_1+1}^{6} \sum_{h_3=h_2+1}^{7} (\beta_{h_1 h_2 h_3}^*)^2 )</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>( \sum_{j=1}^{35} Y_j^2 (n) j )</td>
</tr>
</tbody>
</table>

If the model is simplified to involve only general and bi-specific mixing effects, the yield equation becomes:

\[
Y_{(3)} j = \mu_{(3)} + \sum_{h_1=1}^{7} I_{3j}(h_1)(\alpha_{h_1} + \sum_{h_3=h_1+1}^{7} I_{3j}(h_2)\beta_{h_1 h_3}) + \epsilon(n) j . \tag{15}
\]

The analysis of variance, Table 4, is appropriate when all the units in \( D_{35} \) are observed. The line labeled "Tri-specific Mixing Effect" is now relabeled "Residual".

Seven agents and three agents per object is one of the situations where a BIB design which is minimal exists. There are \( \binom{7}{2} = 21 \) independent parameters, so the BIB design must be irreducible and contain 21 blocks of size three. Such a design is easily found by constructing two BIB designs of size 7 which have no blocks in common. The remaining 21 of the \( \binom{7}{3} = 35 \) possible different blocks form an irreducible BIB design. One such design is:
The parameters of the model can be estimated by inverting the \( N \) matrix in the equation \( Y = NB + \varepsilon \). From this, \( \hat{\beta} = N^{-1}Y \) and the original parameters can be estimated as in the proof of Theorem 1.

An equivalent alternative method of estimation first requires the observation of two facts. If seven blocks form a BIB design and one of the seven is removed, the expected value of the sum of the remaining six blocks is \( 7\mu(3) \) minus the expected value of the missing block. If block \( Y(3)_j \) is missing from the design \( D_{21} \), it is only possible to construct one BIB design of size seven containing \( Y(3)_j \) and six blocks from \( D_{21} \). For example, \( Y(3)_1 \) is not in \( D_{21} \). \( D_{71} \) is the only BIB which can be constructed using six blocks from \( D_{21} \) and \( Y(3)_1 \).

\[
\begin{array}{cccccccccccccccccccc}
& j & 1 & 12 & 13 & 20 & 25 & 27 & 30 \\
\hline
D_{21} & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 2 & 2 & 2 & 2 & 3 & 3 & 3 & 3 & 4 & 4 & 4 & 5 \\
& 2 & 2 & 2 & 3 & 3 & 3 & 4 & 4 & 5 & 3 & 3 & 4 & 4 & 6 & 4 & 5 & 6 & 5 & 5 & 6 \\
& 5 & 6 & 7 & 4 & 5 & 7 & 6 & 7 & 6 & 4 & 5 & 6 & 5 & 7 & 7 & 6 & 7 & 7 & 7 & 7 \\
\end{array}
\]

\[
E(Y(3)_1 + Y(3)_{12} + Y(3)_{13} + Y(3)_{20} + Y(3)_{25} + Y(3)_{27} + Y(3)_{30})
\]

\[
= 7\mu(3) + 3 \sum_{h_1=1}^{7} \alpha_{h_1} + \sum_{h_1=1}^{6} \sum_{h_2=h_1+1}^{7} \beta_{h_1} h_2 = 7\mu(3).
\]

So, \( E(Y(3)_{12} + Y(3)_{13} + Y(3)_{20} + Y(3)_{25} + Y(3)_{27} + Y(3)_{30}) \)

\[
= 7\mu(3) - E(Y(3)_1) = 7\mu(3) - \mu(3) - \alpha_1 - \alpha_2 - \alpha_3 - \beta_{12} - \beta_{13} - \beta_{23}.
\]
It is now possible to estimate all of the missing $Y(3)_j$'s.

$$D\left( \sum_{Y(3)_j \in D_{21}} Y(3)_j \right) = 21\mu(3) + 9 \sum_{h_1=1}^{7} \alpha_{h_1} + 3 \sum_{h_1=1}^{6} \sum_{h_2=h_1+1}^{7} \beta_{h_1} \beta_{h_2} = 21\mu(3).$$

This means $$\mu(3) = \frac{1}{21} \sum_{Y(3)_j \in D_{21}} Y(3)_j.$$  

It is now possible to estimate all of the missing $Y(3)_j$'s.

$$Y(3)_1 = \frac{1}{3} \sum_{Y(3)_j \in D_{21}} Y(3)_j - Y(3)_{12} - Y(3)_{13} - Y(3)_{20} - Y(3)_{25} - Y(3)_{27} - Y(3)_{30}.$$  

The remaining thirteen blocks which do not appear in $D_{21}$ are estimable using the following BIB designs:

<table>
<thead>
<tr>
<th>$D_{72}$</th>
<th>$D_{719}$</th>
<th>$D_{718}$</th>
<th>$D_{721}$</th>
<th>$D_{710}$</th>
<th>$D_{722}$</th>
<th>$D_{731}$</th>
<th>$D_{714}$</th>
<th>$D_{734}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>2 9 13 17 25 27 33</td>
<td>J</td>
<td>4 7 12 19 20 27 35</td>
<td>J</td>
<td>5 6 12 16 21 17 31 33</td>
<td>J</td>
<td>5 7 11 16 23 31 33</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>1 1 1 2 2 3 4</td>
<td></td>
<td>1 1 1 2 2 3 4 5</td>
<td></td>
<td>1 1 1 2 2 3 4 5 6</td>
<td></td>
<td>1 1 1 2 2 3 4 5 6 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 3 5 3 6 4 5</td>
<td></td>
<td>2 3 4 4 6 5 6 7</td>
<td></td>
<td>2 3 4 4 6 5 6 7 8</td>
<td></td>
<td>2 3 4 4 6 5 6 7 8 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 7 6 5 7 6 7</td>
<td></td>
<td>6 5 7 5 7 6 7 8</td>
<td></td>
<td>6 5 7 5 7 6 7 8 9</td>
<td></td>
<td>6 5 7 5 7 6 7 8 9 10</td>
<td></td>
</tr>
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<td></td>
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<table>
<thead>
<tr>
<th>$D_{78}$</th>
<th>$D_{732}$</th>
<th>$D_{714}$</th>
<th>$D_{734}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>3 8 12 16 25 30 32</td>
<td>J</td>
<td>4 9 10 17 22 27 35</td>
</tr>
<tr>
<td></td>
<td>1 1 1 2 2 3 4</td>
<td></td>
<td>1 1 1 2 2 3 4 5</td>
</tr>
<tr>
<td></td>
<td>2 3 4 3 6 5 5</td>
<td></td>
<td>2 3 4 3 6 5 6 7</td>
</tr>
<tr>
<td></td>
<td>5 6 7 4 7 6 7</td>
<td></td>
<td>6 7 5 5 7 6 7 8</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>
Using the fourteen estimated $Y_{(3)j}$'s and the observed $Y_{(3)j}$'s from $D_{21}$, the formulas for estimating parameters which were used with $D_{35}$ may again be used. This results in the following vector of estimates:
Table 5 is the analysis of variance table for $D_{21}$. The indicator function $I_{D_{21}}(h_1 h_2 h_3)$ is one if the block $(h_1 h_2 h_3)$ is in $D_{21}$ and zero otherwise.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean $(\mu_{(3)})$</td>
<td>1</td>
<td>$21(\mu^2_{(3)})$</td>
</tr>
<tr>
<td>General Mixing Effect $(\alpha_{h})$</td>
<td>5</td>
<td>$\sum \sum I_{D_{21}}(h_1 h_2 h_3)(\alpha_{h_1}^* + \alpha_{h_2}^* + \alpha_{h_3}^*)^2$</td>
</tr>
<tr>
<td>Bi-specific Mixing Effect $(\beta_{h_1 h_2})$</td>
<td>14</td>
<td>$\sum \sum I_{D_{21}}(h_1 h_2 h_3)(\beta_{h_1 h_2}^* + \beta_{h_1 h_3}^* + \beta_{h_2 h_3}^*)^2$</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>$\sum \sum \sum I_{D_{21}}(h_1 h_2 h_3)$</td>
</tr>
</tbody>
</table>

$h_1 = 1, h_2 = h_1 + 1, h_3 = h_2 + 1$
VII. Appendix

Minimal treatment designs for estimating bi-specific effects: \( v \) = number of agents; \( n \) = number of agents per treatment.

<table>
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<th>( v )</th>
<th>( n )</th>
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<tr>
<td>7</td>
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<td>275</td>
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- 51 -
<table>
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<th>19</th>
<th>28</th>
<th>42</th>
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VIII. References


