RESPONSE SURFACE METHODS FOR ASSESSING SYNERGISTIC EFFECTS OF THREE OR MORE DRUGS

BY JOHN OLEYNICK

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ABSTRACT OF THE DISSERTATION

Response surface methods for assessing synergistic effects of three or more drugs

by John Oleynick

Dissertation Director: Professor Yong Lin

Drug synergy occurs when two or more drugs are used in a combination therapy, and the total effect of the drugs is more than would be expected based on their individual effects. Although numerous methods exist for evaluating synergy between two drugs, few have been developed for three or more drugs, and most of them have some significant drawbacks.

This dissertation extends a number of two-drug synergy methods to accommodate three or more drugs. First, the method of Plummer and Short [27] is extended to three or more drugs, which is an appropriate method for cases where only global synergy may be present. Next, the parametric method of Kong and Lee [19] is extended, which is appropriate for cases where local synergy may be present. Finally, the semiparametric method of Kong and Lee [20] is extended to three or more drugs, which is an appropriate method for cases where either local synergy may be present or where the model assumptions of the extended Plummer and Short method and the extended Kong and Lee methods are not met.

For each method, synergy models are presented for the case of n drugs; the models are then implemented and evaluated using simulated data for the case of three drugs, and their goodness of fit is evaluated using simulations.

When more than two drugs are used in a combination, there is an additional complexity not present in 2-drug synergy: determining whether any detected synergy is between all n drugs or only a subset of the drugs. All of the new methods presented in this dissertation are able to make that distinction.

Preface

This dissertation is organized as follows.

Chapter 1 briefly describes the background of drug synergy, the motivating example, and the research objectives and specific aims.

Chapter 2 discusses a number of response surface methods for assessing synergy in two drugs, and existing methods for assessing synergy in three drugs.

Chapter 3 describes a formal model for synergy, the Loewe Additivity Model [24, 21].

Chapter 4 describes an extension to n drugs of the two-drug method of Plummer and Short[27]. An implementation of the method is created for three drugs to evaluate the performance of the extended method.

Chapter 5 describes an extension to n drugs of the two-drug parametric method of Kong and Lee[19]. An implementation of the method is created for three drugs to evaluate the performance of the extended method.

Chapter 6 describes an extension to n drugs of the two-drug semiparametric method of Kong and Lee[20]. An implementation of the method is created for three drugs to evaluate the performance of the extended method.

Chapter 7 presents a summary and discussion of the dissertation, and proposes directions for future research.

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Chapter 1

Introduction

1.1 Background

When two or more drugs are used in a combination therapy, it is possible that the total effect of the drugs together may be more or less than would be expected based on their individual effects alone. In some situations, this has the potential to provide valuable benefits. In cancer treatments, where the individual drugs often have toxic side effects at higher doses, a combination therapy that is more effective than expected allows the individual drugs to be given at lower doses, yet still be effective [10, 17]. In other situations, this may be dangerous. For patients receiving treatment for multiple conditions, the combination of treatments may render one or more of the individual treatments ineffective or toxic. The potential for this is especially great in the elderly, where 76% use two or more prescription drugs and 37% use five or more [14]. This phenomenon of the total effect of the drugs being different than would be expected is referred to as "synergy".

The formal definition of the term "synergy" refers to a drug interaction where the response of the combination of drugs is better than would be expected based on the drugs' individual responses. Cases of interactions where the response is worse than would be expected are referred to as "antagonism". Cases where there is no interaction between the drugs, and the response is the same as would be expected are referred to as "additive". The term "synergy" is also used generically to refer to statistical methods that identify and quantify interactions between drugs or other treatments.

Research in synergy goes back at least to the 1920's and 1930's, for applications in pharmacology and in agricultural pesticides [24, 4]. Most research has focused on synergy between only two drugs and a number of methods are now available to identify

synergy between two drugs, but little research has been done on synergy between three or more drugs. Many authors suggest that their methods can be easily extended to three or more drugs, but few have actually done that, and most existing extensions have one or more significant drawbacks.

This thesis proposes extensions to a number of existing 2-drug synergy analysis methods so that the extended methods can identify synergy between 3 drugs, and could be further extended to more than 3 drugs.

1.2 Motivating example

A study was conducted to investigate the potential synergistic effect of all-trans retinoic acid (RA), 1α,25-dihydroxyvitamin D_3 (VD_3), and sodium butyrate (NaB) on 12-O-tetradecanoylphorbol-13-acetate (TPA) induced differentiation in a human promyelocytic leukemia cell line, HL-60. In an initial study, HL-60 cells were treated once with different concentrations of TPA (0.1, 1, 10, and 100 ng/ml) for 48 hours, and the effects of TPA on growth and differentiation of HL-60 cells were assessed by scoring the number of viable cells and the number of adherent cells. In subsequent studies the dose of 0.1ng/ml of TPA was chosen to be studied for potential synergistic effects of TPA, RA, VD_3, and NaB on differentiation in cultured HL-60 cells. In the subsequent studies, doses of 1, 10, 100, 1000, and 10000 nM of RA were used alone and with TPA, doses of 0.1, 1, and 10 nM of VD_3 were used alone and with TPA; and doses of 1, 10, 100, and 1000 nM of NaB were used alone and with TPA. Furthermore a "cocktail" combination of TPA, RA, VD_3 and NaB was also studied. The effects of the treatments on the growth and differentiation of HL-60 cells were assessed by scoring the number of viable cells and the number of adherent cells. Table 1.1 shows the mean number of viable cells and adherent cells for the various treatment combinations.

As the table shows, the two-drug combinations generally have fewer viable cells and more adherent cells than the individual drugs alone. In turn, the three-drug combinations that include TPA generally have fewer viable cells and more adherent cells than the two-drug combinations, while the four-drug combination has the fewest viable cells

Table 1.1: Mean Treatment Results from HL-60 Differentiation Study.

Treatment	Viable Cells ($\times 10^6$)	Adherent Cells
Control	1.590	1.000
Ethanol	1.598	0.800
TPA 0.16 nM	1.421	55.883
VD_3 0.1 nM	1.565	2.017
RA 200 nM	1.323	2.300
NaB 450 μM	1.346	2.083
TPA 0.16 nM + VD_3 0.1 nM	1.456	86.900
TPA 0.16 nM + RA 200 nM	1.175	111.950
TPA 0.16 nM + NaB 450 μM	1.247	89.333
TPA 0.16 nM + VD_3 0.1 nM + RA 200 nM	1.181	181.383
TPA 0.16 nM + VD_3 0.1 nM + NaB 450 μM	1.209	111.250
TPA 0.16 nM + RA 200 nM + NaB 450 μM	1.223	138.817
VD_3 0.1 nM + RA 200 nM + NaB 450 μM	1.313	4.883
TPA 0.16 nM + VD_3 0.1 nM + RA 200 nM + NaB 450 μM	1.060	242.300

and the most adherent cells.

But a simple improvement by a combination of drugs doesn't necessarily mean there is a synergistic relationship between the drugs, the relationship could just be additive, where the amount of improvement is what would be expected based on the individual drugs alone. A more detailed analysis is necessary to confirm that synergy is really present.

An analysis of the two-drug combinations found a number of cases of synergy [23], but the three and four-drug combinations could not be analyzed because no adequate statistical methods were available. In order to determine whether synergy is present in the three and four-drug combinations new statistical methods must be developed.

1.3 Research objectives

1.3.1 Objectives

The objective of this dissertation is to develop statistical methods to evaluate drug synergy for three or more drugs. A review of available methods to assess synergy with

three or more drugs finds only a few candidates, each of which has drawbacks. But there are a large number of methods for assessing two drug synergy. This thesis will extend a number of 2-drug synergy methods to handle three or more drugs, without the drawbacks of the current three-drug methods.

This dissertation will focus on three methods in particular as candidates to be extended to support three or more drugs: the method of Plummer and Short [27], the parametric method of Kong and Lee [19], and the semiparametric method of Kong and Lee [20].

All three of these methods model the dose-response relationship using a response surface. Using the response surface, it is possible to make inferences about intermediate doses that were not tested, in addition to making inferences about the doses that were tested.

The method of Plummer and Short is the simplest of the three methods, but also requires the most assumptions to be met. It assumes each individual drug has a (possibly transformed) response linearly related to the log of the dose. It does not require that the relative potency of one drug to another be constant, but does assume that the relative potency is log-linearly related to the dose. It also assumes that any synergy or antagonism is present at all combinations of non-zero doses, and that the strength of the synergy or antagonism is the same at all of those doses.

The parametric method of Kong and Lee extends the method of Plummer and Short, removing one of its limitations. Because the Plummer and Short method assumes that synergy or antagonism is the same at all non-zero combination doses, in essence it assumes that synergy or antagonism is "global" across all dose combinations. The parametric method of Kong and Lee drops this assumption and allows for "local" regions of synergy or antagonism, so the strength of the synergy may vary from one region to another, and the "direction" may even change from synergy in one region to antagonism in another.

The semiparametric method of Kong and Lee also allows "local" synergy and antagonism, and does so even more flexibly than their parametric method. The parametric method allows for local synergy using a polynomial function of the dose levels, but a

single polynomial function is used to model the relationship for all dose levels. The semiparametric method instead uses thin plate splines (the three-dimensional equivalent of a two-dimensional cubic spline) to model the synergy, allowing for much more flexibility. In addition to the added flexibility from the thin plate splines, the Kong and Lee semiparametric method also allows more flexibility for the dose-response relationship of the individual drugs. Instead of only allowing a linear relationship between log-dose and the (possibly transformed) response, the semiparametric method also allows a linear relationship between the untransformed dose and the (possibly transformed) response.

1.3.2 Specific aims

The specific aims of this dissertation are to extend the 2-drug synergy methods described above to handle three or more drugs.

Specifically, the aims are:

1. Extend the Plummer and Short method to three or more drugs.

2. Extend the Kong and Lee parametric method to three or more drugs.

3. Extend the Kong and Lee semiparametric method to three or more drugs.

For each method a model will be proposed that can accommodate n drugs. The model will then be evaluated in detail by implementing the model for the case of three drugs, and evaluating its estimation of the response surface and its goodness of fit.

Chapter 2

Literature Review

2.1 Overview

The literature on drug synergy is extensive. This chapter will not provide an exhaustive review, but will only focus on research that provides a basis for this thesis to extend. In addition to the three methods mentioned in the previous chapter, this chapter will also review the method of Finney [9], which was a precursor to the method of Plummer and Short, and still serves as the foundation for the other response surface methods described here.

2.2 Finney's Method

Some of the earliest work in using response surface models for synergy was done by Finney [9]. Like the earlier work of Bliss [4], this was done in the area of insecticides and fungicides so the focus is on poisons and toxicity.

Finney used Bliss's terminology and classification of joint toxic action into 3 types: "similar joint action", "independent joint action", and "[similar] synergistic action". Similar joint action refers to the case where the poisons or drugs operate "similarly [and] any quantity of one constituent can be replaced by a proportionate amount of any other without disturbing the potency". Independent joint action refers to cases where "the mortalities, not the doses, are additive", which "may occur with a mixture whose constituents produce their toxic effect in entirely different ways, as, for example, a mixture of two insecticides of which one is a stomach poison and the other a contact poison." Bliss had defined synergy as "characterized by a toxicity greater than that predicted from experiments with the isolated constituents", but one of Finney's goals

was to provide a more exact definition.

In similar joint action, the regression lines of the response (mortality probits in Finney's examples) on log doses are parallel. In this case, the regression lines for two poisons can be written as:

$$Y_1 = a_1 + b \log \lambda \tag{2.1}$$

$$Y_2 = a_2 + b \log \lambda \tag{2.2}$$

where λ is the dose.

The relative potency is defined as "the ratio of equally effective doses", and in Finney's work is constant at all doses. Suppose dose x_1 of the first poison has the same response Y as dose x_2 of the second poison. Then the relative potency of the second to the first is defined as $\rho = x_1/x_2$. Under the assumptions in equations (2.1) and (2.2), it can be shown that $\log \rho = (a_2 - a_1)/b$.

So any dose of the second poison can be converted to an equivalent dose of the first poison by multiplying it by ρ. For a given dose λ_2 of the second poison, its response can be predicted by converting it to an equivalent dose of the first poison, and then using equation (2.1):

$$Y = a_1 + b \log (\rho \lambda_2) .$$

Consequently, in a mixture of the two poisons, with λ_1 of the first poison and λ_2 of the second poison, the second poison can be replaced by its equivalent amount of the first poison, $\rho\lambda_2$, and under the assumption of similar action the response for the mixture is given by:

$$Y = a_1 + b \log (\lambda_1 + \rho \lambda_2) . \tag{2.3}$$

The relationship is the same if the amounts of the two poisons are expressed as proportions of the total dose of the mixture. If λ is the total dose of both poisons, and the proportions of the two poisons in the mixture are π_1 and π_2 respectively, then:

$$Y = a_1 + b \log (\pi_1 + \rho \pi_2) \lambda.$$

or, equivalently, if $x = log\lambda$:

$$Y = a_1 + b \log (\pi_1 + \rho_2 \pi_2) + bx. \tag{2.4}$$

Finney uses (2.4) as the basis for the model of synergy he proposes:

$$Y = a + b\log\left(\pi_1 + \rho\pi_2 + \kappa\sqrt{\rho\pi_1\pi_2}\right) + bx. \tag{2.5}$$

As in (2.4), x is the log of the total dose of the mixture, π_1 and π_2 are the proportions of the two poisons in the mixture, and ρ is the relative potency of the second poison relative to the first. The new term in this equation is κ, which Finney refers to as the "coefficient of synergism". The difference between this equation and the original is the additional term $\kappa\sqrt{\rho\pi_1\pi_2}$. If $\kappa = 0$, then the additional term is 0 and equation (2.5) is identical to equation (2.4), so there is only joint similar action between the two poisons. If κ is positive, then the response is greater than would be predicted by the individual responses alone, so there is synergy between the two poisons. If κ is negative, then the response is less than would be predicted, which is antagonism.

Finney offers no explanation for the choice of the geometric mean here. Presumably any monotonic function of π_1 and $\rho\pi_2$ would work although the interpretation of κ might be different. One advantage of the geometric mean over the arithmetic mean is that it takes on the value of 0 whenever either π_1 or π_2 is 0, reflecting the fact that the model reduced to a model with only one poison alone. Another possible advantage of the geometric mean over other monotonic functions is that it gives equal weight to both components of the mixture.

Finney's proposed model addresses a number of deficiencies he found in two of Bliss's early models of synergy. It is not clear why Finney does not address Bliss's independence model, because Finney's paper does reference Bliss's later work, which included the independence model. Finney does not appear to have been aware of Loewe's model [24]. Perhaps that is because Loewe's work was originally published in a German language publication, in 1926, and he did not publish in English language publications until much later, in 1953, while Finney published his proposed model in 1942.

Finney described a method for estimating κ in Equation (2.5) using the LD_{50}s of the individual components and their combinations, but that will not be described here. Although it was useful at the time it was originally proposed, now the parametrization

in (2.5) can be estimated directly using nonlinear regression.

Although he did not fully develop it, Finney stated that the concepts here could be "easily" extended to three or more poisons, as long as the assumption was still met that the regression lines of the (possibly transformed) response on log dose for all individual poisons and their mixtures were parallel. He extended equation (2.3) for the case of three poisons. In this case, there are three poisons with doses λ_1, λ_2, and λ_3, respectively, with the second and third poisons having potencies of ρ_2 and ρ_3 relative to the first. If the poisons have similar action, a dose of $\lambda_1 + \rho_2\lambda_2 + \rho_3\lambda_3$ of the first poison should be equivalent to the mixture, so the regression line of the mixture would then be:

$$Y = a_1 + b \log\left(\lambda_1 + \rho_2\lambda_2 + \rho_3\lambda_3\right).$$

2.3 Plummer and Short's Method

The work of Plummer and Short [27] extends and generalizes the work of Finney [9]. One of the key assumptions of Finney's model is that the relative potency of one drug to the other must be constant. This is reflected in the requirement that the (log) dose-response curves of the two drugs must be parallel. Although Finney mentioned the possibility of the curves not being parallel, he did not attempt to "unravel the complexities". The model of Plummer and Short [27] addresses the additional complexities of non-constant relative potencies, and is thus able to relax that requirement somewhat.

The Plummer and Short model begins with a similar assumption as Finney, that the individual drugs have straight log dose-response curves. Using a notation slightly different from Finney, the response of one drug, Drug A, is described as:

$$Y = \beta_0 + \beta_1 \log\left(A\right)$$

where Y is the response, A is the dose of Drug A, and β_0 and β_1 are respectively the intercept and slope of the linear log dose-response curve.

Plummer and Short first restate Finney's model in their own notation by assuming that there is a second drug, Drug B, whose log dose-response curve is parallel to that of Drug A, and which has a constant relative potency of P to Drug A. If the drugs have

an additive effect, then the response for a combination of Drug A and Drug B can be written as:

$$Y = \beta_0 + \beta_1 \log\left(A + P * B\right)$$

where B is the dose of Drug B. In this case, $P*B$ is the dose of Drug A that is equivalent to the dose B of Drug B. If the drugs do not have an additive effect, then their response can be modeled using Finney's model, expressed in Plummer and Short's notation as:

$$Y = \beta_0 + \beta_1 \log\left(A + P * B + \beta_2 \left(A * P * B\right)^{1/2}\right)$$

where β_2 is the coefficient of synergism.

If the relative potency of the two drugs is not constant, then the log dose-response curves will not be parallel. But, as long as both curves are linear, the logarithm of the relative potency at any dose level is linearly related to the log dose of either drug. This is the case that Plummer and Short's method can handle which Finney's method could not.

If the log of the relative potency at any dose level is linearly related to the log dose of either drug, then:

$$\log\left(P\right) = \beta_2 + \beta_3 \log\left(B\right)$$

where β_2 and β_3 are constants.

When both drug A and drug B are present, Plummer and Short treat a combination of the two drugs as a dose B' of drug B alone, in which some amount of drug B has been replaced by an amount of drug A with an equivalent effect:

$$B' = B + A/P$$

In this case, Plummer and Short use the relative potency at the dose B' level, so:

$$\log\left(P\right) = \beta_2 + \beta_3 \log\left(B'\right) \tag{2.6}$$

The Plummer and Short model is then defined as:

$$Y = \beta_0 + \beta_1 \log\left(A + P * B + \beta_4 \left(A * P * B\right)^{1/2}\right)$$

where P is defined as in (2.6), and B' is the solution to:

$$B' = B + A * e^{-\beta_2 - \beta_3 \log(B')} \quad (= B + A/P) \tag{2.7}$$

The model can also be equivalently expressed as:

$$Y = \beta_0 + \beta_1 \log\left(A + B * e^{\beta_2 + \beta_3 log(B')} + \beta_4 \left(A * B * e^{\beta_2 + \beta_3 log(B')}\right)^{1/2}\right)$$

where B' is the solution to (2.7). This model can be fitted using iterative methods for nonlinear regression, solving (2.7) numerically at each iteration for each dose combination to determine B'.

In the model, β_0 and β_1 represent the intercept and slope of the log dose-response curve for drug A alone. β_2 and β_3 describe the difference between the drug B and drug A log dose-response curves, with β_2 corresponding to the "horizontal" difference between the lines and β_3 corresponding to the difference of slopes. If the log dose-response curves are parallel, then $\beta_3 = 0$. β_4 represents the coefficient of synergy in this model, with positive values indicating synergy.

The presence of synergy can be tested by fitting full and reduced models with and without the β_4 term. The assumption of parallel log dose-response curves can be tested by fitting full and reduced models with and without the β_3 term.

2.4 Kong and Lee's Parametric Method

While the model of Plummer and Short [27] generalized the work of Finney [9] to remove the restriction of constant relative potency between the two drugs, it still used a single parameter to model synergy. This forced the model to treat synergy as being constant at all dose levels and combinations, so it could not accurately model a case where some dose combinations were synergistic, while others were additive or were even antagonistic, or where different combination treatments had different degrees of synergy. To solve this problem, Kong and Lee [19] proposed a more general parametric model that allowed for regions of "local" synergy or antagonism, which they called the Generalized Response Surface (GRS) model.

The GRS model uses the Loewe additivity model [24, 21] as a starting point. The Loewe additivity model is specified as:

$$\frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}} = 1 \tag{2.8}$$

where d_1 and d_2 are the doses of drugs 1 and 2 respectively in the mixture, and y is the additive effect at the combination treatment of (d_1, d_2). $D_{y,1}$ and $D_{y,2}$ are the doses of drug 1 and drug 2 respectively that are required to produce effect y when each drug is used alone. The function $F_1(D_1)$ denotes the dose-response curve for drug 1 alone while the function $F_2(D_2)$ denotes the curve for drug 2 alone. Given the inverses of the dose-response functions, $F_1^{-1}(y)$ and $F_2^{-1}(y)$, the predicted additive effect y for a combination of two doses can be calculated by replacing $D_{y,1}$ and $D_{y,2}$ by $F_1^{-1}(y)$ and $F_2^{-1}(y)$ respectively, and then solving equation (2.8).

Equation (2.8) can be re-written as:

$$d_1 + d_2 \frac{D_{y,1}}{D_{y,2}} = D_{y,1} \tag{2.9}$$

where $\frac{D_{y,1}}{D_{y,2}}$ is the relative potency of drug 2 to drug 1, which can be notated as $\rho\,(y)$.

The GRS model assumes that the individual log dose-response curves are linear and of the form:

$$\begin{aligned} Y_1 &= \beta_0 + \beta_1 \log D_{Y,1}, \\ Y_2 &= \alpha_0 + \alpha_1 \log D_{Y,2} \end{aligned} \tag{2.10}$$

If the relative potency ρ is constant, then the predicted additive effect for the mixture, from (2.9) and (2.10), is:

$$Y = \beta_0 + \beta_1 \log\left(d_1 + \rho d_2\right)$$

Adding a single parameter to model the synergy relationship gives the Finney model:

$$Y = \beta_0 + \beta_1 \log\left(d_1 + \rho d_2 + \kappa\left(d_1 \rho d_2\right)^{\frac{1}{2}}\right) \tag{2.11}$$

where κ indicates whether the relationship is additive ($\kappa = 0$), synergistic ($\kappa > 0$), or antagonistic ($\kappa < 0$).

The constant relative potency requirement can then be relaxed as follows. Let $Y_1 = Y_2 = y$, so:

$$\begin{aligned}
\beta_0 + \beta_1 \log D_{y,1} =& \alpha_0 + \alpha_1 \log D_{y,2} \\
\beta_1 \log D_{y,1} =& \alpha_0 - \beta_0 + (\alpha_1 - \beta_1) \log D_{y,2} + \beta_1 \log D_{y,2} \\
\beta_1 \log \frac{D_{y,1}}{D_{y,2}} =& \alpha_0 - \beta_0 + (\alpha_1 - \beta_1) \log D_{y,2} \\
\log \frac{D_{y,1}}{D_{y,2}} =& \frac{\alpha_0 - \beta_0}{\beta_1} + \frac{\alpha_1 - \beta_1}{\beta_1} \log D_{y,2} \\
\log \rho(y) =& \frac{\alpha_0 - \beta_0}{\beta_1} + \frac{\alpha_1 - \beta_1}{\beta_1} \log D_{y,2}
\end{aligned}$$

Define $\gamma_1 = \frac{\alpha_0 - \beta_0}{\beta_1}$ and $\gamma_2 = \frac{\alpha_1 - \beta_1}{\beta_1}$ and the relative potency can then be expressed as:

$$\rho(y) = \exp(\gamma_1 + \gamma_2 \log D_{y,2})$$

As before, $D_{y,2}$ is the amount of drug 2 alone that has the same effect as the mixture, assuming additivity, that is $D_{y,2} = \rho(y)^{-1} d_1 + d_2$. But given one of the two, $D_{y,2}$ and y are uniquely determined, so y can be suppressed and the relative potency at a dose (d_1, d_2) can be calculated by solving $\rho = \exp(\gamma_1 + \gamma_2 \log D_2)$ subject to $D_2 = \rho^{-1} d_1 + d_2$.

Relaxing the requirement of a constant ρ in this manner, combined with the Finney model in (2.11) results in the Plummer and Short model. But the model still suffers from a single parameter to assess the synergy.

The solution of the GRS model is to replace the single synergy parameter κ by a function of the doses, the relative potency parameters, and multiple synergy parameters as follows:

$$Y = \beta_0 + \beta_1 \log\left(d_1 + \rho d_2 + f(d_1, d_2; \gamma, \kappa)(d_1 \rho d_2)^{\frac{1}{2}}\right) \tag{2.12}$$

where the function $f(d_1, d_2; \gamma, \kappa)$ is able to capture any local synergy or antagonism, not just global synergy or antagonism. In their paper Kong and Lee define the function as:

$$\begin{aligned}
f(d_1, d_2; \gamma, \kappa) =& \kappa_0 + \kappa_1 d_1^{\frac{1}{2}} + \kappa_2 (\rho d_2)^{\frac{1}{2}} + \kappa_3 d_1 \\
&+ \kappa_4 \rho d_2 + \kappa_5 (d_1 \rho d_2)^{\frac{1}{2}}
\end{aligned} \tag{2.13}$$

where f is a function of d_1 and d_2, the γ parameters model the relative potency of ρ, and the κ parameters are the coefficients of the quadratic function and model the

synergy relationship. If $\kappa_1 = \kappa_2 = \kappa_3 = \kappa_4 = \kappa_5 = 0$, then the model reduces to the Plummer and Short model.

When using the GRS model, Kong and Lee suggest that the first analysis step is to determine whether the GRS model significantly improves the Plummer and Short model by testing $H_0 : \kappa_1 = \kappa_2 = \kappa_3 = \kappa_4 = \kappa_5 = 0$ against $H_1 : \kappa_i \neq 0$ for any i where $i = 1, ..., 5$ using an F-statistic:

$$F = \frac{\left(\mathrm{RSS}_{\mathrm{P-S}} - \mathrm{RSS}_{\mathrm{full}}\right)/q}{\mathrm{RSS}_{\mathrm{full}}/\left(n-p\right)}$$

with $q = 5$ and $n - p$ degrees of freedom, where n is the number of observations and $p = 10$ is the number of parameters in the model (2.12) and (2.13), $\mathrm{RSS}_{\mathrm{full}}$ is the residual sum of squares from the GRS model, and $\mathrm{RSS}_{\mathrm{P-S}}$ is the residual sum of squares of the Plummer and Short model. Kong and Lee caution that the F test here assumes that the responses on the Y-scale are normally distributed, and that the assumption should be checked with residual plots or some other method.

If the GRS model does significantly improve the Plummer and Short model, the next step is to remove any unnecessary terms, using a backward elimination procedure and the Akaike Information Criterion (AIC).

The procedure begins by fitting the full GRS model and calculating the AIC. Next, a term to be removed is selected. The candidate terms are all γ, β and higher-order terms of κ's that have a p-value greater than a defined level of significance α, such as $\alpha = 0.10$. The term with the smallest absolute t-value of the candidates is removed from the model, with the restriction that no lower-order terms are removed until after the corresponding higher-order terms have been removed. After the term is removed, a new model is fitted and the AIC of the new model is calculated. The process continues until all γ, β and higher-order terms of κ's left in the model have a p-value less α, or until the AIC value increases.

The synergy at a specified combination dose (d_1, d_2) can be determined by determined by the sign and magnitude of the polynomial function $f\left(d_1, d_2; \gamma, \kappa\right)$. Because γ and κ are estimated, their asymptotic properties follow the standard results from

a nonlinear regression. For each combination dose (d_1, d_2), the variance of the estimated polynomial $f(d_1, d_2; \gamma, \kappa)$ can be approximated using the delta method by $\widehat{\text{Var}}_f = \left(\frac{\partial f}{\partial(\gamma,\kappa)}\right)' \Sigma \left(\frac{\partial f}{\partial(\gamma,\kappa)}\right)|_{(\gamma,\kappa)=(\hat{\gamma},\hat{\kappa})}$ where

$$\begin{aligned}\frac{\partial f}{\partial(\gamma,\kappa)} &= \left(\frac{\partial f}{\partial\gamma_1}, \frac{\partial f}{\partial\gamma_2}, \frac{\partial f}{\partial\kappa_0}, \frac{\partial f}{\partial\kappa_1}, \frac{\partial f}{\partial\kappa_2}, \frac{\partial f}{\partial\kappa_3}, \frac{\partial f}{\partial\kappa_4}, \frac{\partial f}{\partial\kappa_5}\right)' \\ &= \left(\frac{\partial f}{\partial\rho}\frac{\partial f}{\partial\gamma_1}, \frac{\partial f}{\partial\rho}\frac{\partial f}{\partial\gamma_2}, 1, d_1^{\frac{1}{2}}, (\rho d_2)^{\frac{1}{2}}, d_1, \rho d_2, (d_1\rho d_2)^{\frac{1}{2}}\right)'\end{aligned}$$

with $\frac{\partial f}{\partial\rho} = \frac{1}{2}\kappa_2\left(\frac{d_2}{\rho}\right)^{\frac{1}{2}} + \kappa_4 d_2 + \frac{1}{2}\kappa_5\left(\frac{d_1 d_2}{\rho}\right)^{\frac{1}{2}}$, $\frac{\partial\rho}{\partial\gamma_1} = \frac{\rho^2\left(d_2+d_1\rho^{-1}\right)}{\rho(d_2+d_1\rho^{-1})+\gamma_2 d_1}$, and $\frac{\partial\rho}{\partial\gamma_2} = \frac{\rho^2\left(d_2+d_1\rho^{-1}\right)}{\rho(d_2+d_1\rho^{-1})+\gamma_2 d_1}\log\left(d_2 + d_1\rho^{-1}\right)$. Σ is the estimated covariance matrix of the parameters $(\gamma_1, \gamma_2, \kappa_0, \kappa_1, \kappa_2, \kappa_3, \kappa_4, \kappa_5)$. So the $(1-\alpha)\times 100\%$ upper and lower confidence surfaces for $f(d_1, d_2; \gamma, \kappa)$ can be constructed as:

$$f_{l,u}(d_1, d_2) = \hat{f}(d_1, d_2) \pm t_{\frac{\alpha}{2},n-p}\sqrt{\widehat{\text{Var}}_f(d_1, d_2)},$$

where $t_{\frac{\alpha}{2},n-p}$ is the upper $\frac{\alpha}{2}$ percentile of a t-distribution with $n-p$ degrees of freedom.

2.5 Kong and Lee's Semiparametric Method

While the parametric model of Kong and Lee allowed for regions of local synergy and antagonism, it required that the scaled responses be normally distributed, and involved a model building process to remove insignificant terms from the model. They later proposed a semiparametric model [20] that was more robust to departures from the model assumptions, while still allowing regions of local synergy or antagonism.

2.5.1 The Semiparametric Model

Like their parametric model, the Kong and Lee semiparametric model uses the Loewe additivity model [24, 21] as a starting point. The Loewe additivity model is specified as:

$$\frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}} = 1 \tag{2.14}$$

where d_1 and d_2 are the doses of drugs 1 and 2 respectively in the mixture, and y is the theoretic additive effect at the combination treatment of (d_1, d_2). $D_{y,1}$ and $D_{y,2}$ are the doses of drug 1 and drug 2 respectively that are required to produce effect y

when each drug is used alone. The function $F_1(D_1)$ denotes the dose-response curve for drug 1 alone while the function $F_2(D_2)$ denotes the curve for drug 2 alone. Given the inverses of the dose-response functions, $F_1^{-1}(y)$ and $F_2^{-1}(y)$, the predicted additive effect y for a combination of two doses can be calculated by replacing $D_{y,1}$ and $D_{y,2}$ by $F_1^{-1}(y)$ and $F_2^{-1}(y)$ respectively, and then solving equation (2.14). Kong and Lee denote the predicted additive effect for combination dose (d_1, d_2) as $F_p(d_1, d_2)$.

The Kong and Lee semiparametric model is a two-component model defined as:

$$Y = F_p\left(d_1, d_2\right) + f\left(d_1, d_2\right) \tag{2.15}$$

$F_p(d_1, d_2)$ is the predicted additive effect, as described in the preceding paragraph. The interaction part $f(d_1, d_2)$ is a function that is estimated nonparametrically to capture any departure from additivity, and hence any synergy or antagonism. Because f is a function of the doses, it allows for local synergy and antagonism, not just global synergy or antagonism. If the observed effect at the combination dose (d_1, d_2) is more or less than the predicted effect, then $f(d_1, d_2)$ will be non-zero and there is synergy or antagonism for that combination dose. The model also contains a mean zero random error term that is not shown in the equation.

2.5.2 Fitting the Parametric Part of the Model

The model is fitted in separate steps. First the parametric part is fitted to determine the additive part $F_p(d_1, d_2)$ of the model, and then a nonparametric method is used to find $f(d_1, d_2)$.

The parametric part can be fitted in a number of different ways. Kong and Lee describe methods for two common cases: (1) where the individual drugs have linear dose-response curves (possibly with the response transformed as necessary), and (2) where the individual drugs have linear log dose-response curves (again, possibly with a transformation of the response).

In the first case, the dose-response curves for drugs 1 and 2 can be specified respectively as:

$$Y = g(E) = \beta_0 + \beta_1 D_{y,1}, \tag{2.16}$$

$$Y = g(E) = \beta_0 + \beta_2 D_{y,2} \tag{2.17}$$

Where E is the observed effect at the given dose, and $g(E)$ is a monotonic function, such as $g(E) = \log \frac{E}{1-E}$. The intercepts in (2.16) are the same because the effects should be the same for a dose of zero of either drug. Kong and Lee refer to Y as the transformed effect. Based on the Loewe additivity model, the predicted effect in Y-scale can be calculated from $\frac{d_1}{(Y-\beta_0)/\beta_1} + \frac{d_2}{(Y-\beta_0)/\beta_2} = 1$. The predicted additive response function is then:

$$Y = g(E) = F_p(d_1, d_2) = \beta_0 + \beta_1 d_1 + \beta_2 d_2$$

In the second case, where the individual drugs have linear log dose-response curves, the (possibly transformed) effects can be specified as:

$$Y = g(E) = \beta_0 + \beta_1 log D_{y,1},$$

$$Y = g(E) = \alpha_0 + \alpha_1 log D_{y,2}$$

Here again, E is the observed effect at the given dose, and $g(E)$ is a monotonic function, and Y is the transformed effect. In their earlier work [19], Kong and Lee showed that based on the Loewe additivity model, the predicted additive response function is:

$$Y = g(E) = F_p(d_1, d_2) = \beta_0 + \beta_1 log(d_1 + \beta_2 \rho d_2)$$

where ρ is obtained by solving:

$$\rho = \exp\left(\frac{\alpha_0 - \beta_0}{\beta_1} + \frac{\alpha_1 - \beta_1}{\beta_1} \log\left(\rho^{-1} d_1 + d_2\right)\right)$$

2.5.3 Fitting the Nonparametric Part of the Model

Once the additive part of (2.15) has been estimated, the interaction part, $f(d_1, d_2)$, can be estimated. For most observed dose combinations (d_{1i}, d_{2i}) $(i = 1, \ldots, n)$, f could be estimated by the difference of the observed effect and the predicted additive effect,

$Y_i - \hat{F}_p(d_{1i}, d_{2i})$, where $Y_i = g(E_i)$. But $f(d_1, d_2)$ is used to model the departure from additivity, so it should always be zero when either drug is used alone. To ensure this, Kong and Lee define an indicator function that only has a unit value when both drug doses are non-zero:

$$1_{\{d_1 \neq 0 \ \& \ d_2 \neq 0\}} = \begin{cases} 1 & \text{if } d_1 \neq 0 \ \& \ d_2 \neq 0, \\ 0 & \text{Otherwise.} \end{cases}$$

The nonparametric part $f(d_1, d_2)$ can then be estimated for all dose combinations (d_{1i}, d_{2i}) $(i = 1, \ldots, n)$, by the differences $\left\{Y_i - \hat{F}_p(d_{1i}, d_{2i})\right\} 1_{\{d_{1i} \neq 0 \ \& \ d_{2i} \neq 0\}}$ for $i = 1, \ldots, n$.

Modeling the Nonparametric Part as a Thin Plate Spline

The function $f(d_1, d_2)$ can be estimated by minimizing a penalized residual sum of squares (PRSS):

$$PRSS = \sum_{i=1}^{n} \left(\left(Y_i - \hat{F}_p(d_{1i}, d_{2i}) \right) 1_{\{d_{1i} \neq 0 \ \& \ d_{2i} \neq 0\}} - f(d_{1i}, d_{2i}) \right)^2 + \lambda J(f) \qquad (2.18)$$

where the first term measures the goodness of fit, the third term, $J(f)$, measures the smoothness of the function $f(d_1, d_2)$, and the second term, λ, is a smoothing parameter that measures the trade off between the goodness of fit and the smoothness of the function f.

But the minimizer of $PRSS$ is necessarily a natural thin plate spline [13]. As a thin plate spline, $f(d_1, d_2)$ can be expressed as a linear combination of the radial basis functions:

$$f(d_1, d_2) = \gamma_0 + \gamma_1 d_1 + \gamma_2 d_2 + \sum_{k=1}^{K} v_k \eta \left(\| (d_1, d_2)^T - (\kappa_{1k}, \kappa_{2k})^T \| \right)$$

with the radial basis function:

$$\eta(r) = \begin{cases} \frac{1}{16\pi} r^2 \log r^2 & \text{for } r > 0, \\ 0 & \text{for } r = 0. \end{cases}$$

and with knots, $(\kappa_{1k}, \kappa_{2k})^T$ $(k = 1, \ldots, K)$, which are all of the distinct values of the combination doses $(d_{1i}, d_{2i})^T$ $(i = 1, \ldots, n)$. The distance between any two combination

doses is defined as the Euclidean distance, so the distance between a combination dose and a knot is:

$$\|(d_1, d_2)^T - (\kappa_{1k}, \kappa_{2k})^T\| = \sqrt{(d_1 - \kappa_{1k})^2 + (d_2 - \kappa_{2k})^2}.$$

Kong and Lee define a $K \times K$ matrix $\Omega = \left[\|(\kappa_{1k}, \kappa_{2k})^T - (\kappa_{1k'}, \kappa_{2k'})^T\|\right]_{1 \le k,k' \le K}$, a $K \times 3$ matrix $T^T = [1, \kappa_{1k}, \kappa_{2k}]_{1 \le k \le K}$, and a vector $v = (v_1, \ldots, v_k)^T$. The minimizer of (2.18) then satisfies $J(f) = v^T \Omega v$ and $Tv = 0$. They then define:

$$\begin{aligned} Y_R = [&\left(Y_1 - \hat{F}_p(d_{11}, d_{21})\right) 1_{\{d_{11} \neq 0 \,\&\, d_{21} \neq 0\}}, \ldots, \\ &\left(Y_n - \hat{F}_p(d_{1n}, d_{2n})\right) 1_{\{d_{1n} \neq 0 \,\&\, d_{2n} \neq 0\}}]^T, \end{aligned}$$

$$\begin{aligned} X &= [1, d_{1i}, d_{2i}]_{1 \le i \le n} \in R^{n \times 3}, \\ Z_1 &= \left[\eta\left(\|(d_{1i}, d_{2i})^T - (\kappa_{1k}, \kappa_{2k})^T\|\right)_{1 \le i \le n}\right] \in R^{n \times K}, \end{aligned}$$

and

$$\gamma = (\gamma_0, \gamma_1, \gamma_2)^T.$$

Suppose FG is a QR decomposition of T^T such that F is a $K \times K$ orthogonal matrix, G is a $K \times 3$ upper triangular matrix, and $T^T = FG$. Let F_1 be the first three columns of F, and F_2 be the remaining $K - 3$ columns. Following the argument in Green and Silverman (1994, p. 166), Kong and Lee can then show that $Tv = 0$ if and only if v can be expressed as $F_2\xi$, where ξ is a $K - 3$ vector. The minimizer of (2.18) is then essentially equivalent to minimizing

$$(Y_R - X_\gamma - Z_1 F_2 \xi)^T (Y_R - X_\gamma - Z_1 F_2 \xi) + \lambda \xi^T F_2^T \Omega F_2 \xi. \tag{2.19}$$

Estimating the Thin Plate Spline Using a Mixed Effects Model

In order to minimize (2.19), select the smoothing parameter λ, and estimate the function $f(d_1, d_2)$, Kong and Lee adopted the technique of Ruppert, Wand and Carroll of using a mixed effect model to estimate a thin plate spline [28].

First, define $u = \left(F_2^T \Omega F_2\right)^{\frac{1}{2}} \xi$, where $\left(F_2^T \Omega F_2\right)^{\frac{1}{2}}$ is the matrix square root of $F_2^T \Omega F_2$ (Ruppert et al., 2003, p. 329). Then minimizing (2.19) is equivalent to minimizing

$$(Y_R - X_\gamma - Z_1 u)^T (Y_R - X_\gamma - Z_1 u) + \lambda u^T u. \tag{2.20}$$

where $Z = Z_1 F_2 \left(F_2^T \Omega F_2\right)^{-\frac{1}{2}}$. Expression (2.20) is proportional to the negative exponential part of the joint distribution of Y_R and u under the following model assumption:

$$Y_R = X\gamma + Zu + \epsilon$$

with

$$\begin{pmatrix} u \\ \epsilon \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_u^2 I_{K-3} & 0 \\ 0 & \sigma_\epsilon^2 I_n \end{pmatrix}\right) \tag{2.21}$$

where λ is replaced by $\sigma_\epsilon^2/\sigma_u^2$. The solution of minimizing (2.20) is the same as the best linear unbiased predictor (BLUP) for γ and u in the mixed model (2.21). This solution can be written as

$$\begin{pmatrix} \tilde{\gamma} \\ \tilde{u} \end{pmatrix} = \text{BLUP} \begin{pmatrix} \gamma \\ u \end{pmatrix} = \left(C^T C + \lambda D\right)^{-1} C^T Y_R, \tag{2.22}$$

with $C = [X\ Z]$ and $D = \text{diag}\,(0, 0, 0, 1, \ldots, 1)$, where the number of zeros in the matrix D corresponds to the number of γ_i's $(i = 0, 1, 2)$, and the number of ones corresponds to the number of u_i's $(i = 1, \ldots, K-3)$.

For any combination dose (d_1, d_2), if we denote $f\,(d_1, d_2) = \gamma_0 + \gamma_1 d_1 + \gamma_2 d_2 + Z_0 u$ with $Z_0 = \left[\eta\left(\|(d_1, d_2)^T - (\kappa_{1k}, \kappa_{2k})^T\|\right)\right]_{1 \le k, k' \le K} F_2 \left(F_2^T \Omega F_2\right)^{-\frac{1}{2}}$, then $\tilde{f}\,(d_1, d_2) = \tilde{\gamma}_0 + \tilde{\gamma}_1 d_1 + \tilde{\gamma}_2 d_2 + + Z_0 \tilde{u}$ is the BLUP for $f\,(d_1, d_2)$. Usually σ_ϵ^2 and σ_u^2 are unknown, and so $\lambda = \sigma_\epsilon^2/\sigma_u^2$ is unknown, and $\tilde{\gamma}$ and $\tilde{u}$ are also unknown. If $\hat{\sigma}_\epsilon^2$ and $\hat{\sigma}_u^2$ are the restricted maximum likelihood estimators (REML) σ_ϵ^2 and σ_u^2 in the mixed model (2.21), and $\hat{\lambda} = \hat{\sigma}_\epsilon^2/\hat{\sigma}_u^2$ is used to estimate λ in (2.22), we can then obtain the estimated BLUP for γ and u, $\hat{\gamma}$ and $\hat{u}$. Then the estimated BLUP for $f\,(d_1, d_2)$ is $\hat{f}\,(d_1, d_2) = \hat{\gamma}_0 + \hat{\gamma}_1 d_1 + \hat{\gamma}_2 d_2 + Z_0 \hat{u}$. Especially, the fitted value $\hat{f}\,(d_{1i}, d_{2i})$ $(i = 1, \ldots, n)$ is the i^{th} component of $C\left(C^T C + \hat{\lambda} D\right)^{-1} C^T Y_R$.

2.5.4 Estimating the Variance of the Nonparametric Part

The nonparametric part of the model, $f\,(d_1, d_2)$, which assesses synergy, has now been estimated, but to assess it statistically the variability of $f\,(d_1, d_2)$ must be determined. Kong and Lee believe it would be very difficult to derive a theoretic formula for the variance of $f\,(d_1, d_2)$ in the two-stage estimation framework. Instead they use a bootstrap

method to estimate the variance of $f(d_1, d_2)$ and use that to construct a confidence interval for it.

Kong and Lee considered a bootstrap approximation for a partially linear regression model in a semiparametric framework that Liang et. al. had presented [22], but did not use it because they were concerned that the standard errors of the residuals from estimating the dose-effect curves may be very different than those of the residuals from estimating the function $f(d_1, d_2)$. Instead, they used a wild bootstrap method that could account for the different error structures [8]. The wild bootstrap method doesn't just resample the residuals, it uses a product of each residual and a random number with a mean of zero and standard deviation of 1, which allows the different error structures [15]. Kong and Lee also followed a recommendation by Davison and Hinkley (1997, Section 7.6) to reduce the biases in estimating the standard error for $\hat{f}(d_1, d_2)$. Kong and Lee summarized their procedure as follows:

Step 1. Fit the model based on the original observations, obtain $\hat{f}(d_{1i}, d_{2i})$ and $\hat{\lambda}$, where $\hat{f}(d_{1i}, d_{2i})$ is the i^{th} component of $C\left(C^TC + \hat{\lambda}D\right)^{-1} C^TY_R$.

Step 2. Obtain the residuals from the undersmoothed estimation of $f(d_1, d_2)$, that is, $\hat{\epsilon}_i = Y_i - \hat{F}_p(d_{1i}, d_{2i}) - \hat{f}_{0.5\hat{\lambda}}(d_{1i}, d_{2i})$. Here $\hat{f}_{0.5\hat{\lambda}}(d_{1i}, d_{2i})$ is the i^{th} component of $C\left(C^TC + 0.5\hat{\lambda}D\right)^{-1} C^TY_R$.

Step 3. Generate n i.i.d. (independent and identically distributed) random variables $\epsilon_1^*, \ldots, \epsilon_n^*$ with mean 0 and variance 1, for example, $\epsilon_i^* = -\frac{\sqrt{5}-1}{2}$ with probability $\frac{\sqrt{5}+1}{2\sqrt{5}}$ and $\epsilon_i^* = \frac{\sqrt{5}+1}{2}$ with probability $\frac{\sqrt{5}-1}{2\sqrt{5}}$ (Handle and Marron, 1991).

Step 4. Obtain the fitted value from the oversmoothed estimation of $f(d_1, d_2)$, say, $Y_i^* = \hat{F}_p(d_{1i}, d_{2i}) + \hat{f}_{2\hat{\lambda}}(d_{1i}, d_{2i}) + \hat{\epsilon}_i\hat{\epsilon}_i^*$ for $i = 1, \ldots, n$. Here $\hat{f}_{2\hat{\lambda}}(d_{1i}, d_{2i})$ is the i^{th} component of $C\left(C^TC + 2\hat{\lambda}D\right)^{-1} C^TY_R$.

Step 5. Fit the model using the generated data (d_{1i}, d_{2i}, Y_i^*) $(i = 1, \ldots, n)$, and then obtain the estimated function $f^*(d_1, d_2)$.

Step 6. Repeat step 2 to step 5 B (say, 50) times.

Kong and Lee denote the estimated $f(d_1, d_2)$ in the bth $(b = 1, \ldots, B)$ iteration as

$f^{*b}(d_1, d_2)$, and then estimate the standard deviation of $f(d_1, d_2)$ by:

$$\widehat{SD}^{*B}\left(\hat{f}(d_1, d_2)\right) = \left(\frac{1}{B}\sum_{b=1}^{B}\left(f^{*b}(d_1, d_2) - \hat{f}(d_1, d_2)\right)^2\right)^{\frac{1}{2}},$$

They then construct a $100(1-\alpha)\%$ pointwise confidence interval for $f(d_1, d_2)$:

$$\left[\hat{f}(d_1, d_2) - z_{\frac{\alpha}{2}} \times \widehat{SD}^{*B}\left(\hat{f}(d_1, d_2)\right), \hat{f}(d_1, d_2) + z_{\frac{\alpha}{2}} \times \widehat{SD}^{*B}\left(\hat{f}(d_1, d_2)\right)\right],$$

where $z_{\frac{\alpha}{2}}$ is the upper $\frac{\alpha}{2} \times 100\%$ percentile of the standard normal distribution, and $\hat{f}(d_1, d_2)$ is the estimated BLUP for $f(d_1, d_2)$.

Kong and Lee performed a case study which showed that the estimated variance for $f(d_1, d_2)$ can account for the carry-over errors from estimating the marginal dose-response curves. They performed a simulation study that showed that their bootstrap confidence intervals have good coverage properties. While extending this method to 3 drugs, we discovered some bias issues with the bootstrap samples used to estimate the variance for the confidence intervals; see Appendix C for details.

2.6 Methods for Three Drugs

Although most work in the drug synergy area has been done for the two drug case, some work has been done for three or more drugs. This section will briefly describe that work and mention the benefits and drawbacks of the current approaches.

The Median Effect method of Chou and Talalay [6] can be used with 3 or more drugs. It has been used in oncology studies of 3 drugs [5], and is even available as part of a commercial software package. However, this method has a number of drawbacks. First, it can only analyze experiments conducted using "ray" designs, where each combination of drugs has a constant ratio of doses. Multiple rays can be analyzed separately, but unlike response surface methods, such analyses cannot use the data from the multiple rays to increase their sensitivity. Second, the method cannot identify 3-way synergy above and beyond any 2-way synergy (or, equivalently, using the definitions in Section 3.1, it can only identify 3-way synergy based on Definition I, but not 3-way synergy based on Definition II). And most important, the method as described and used in the cited references does not use statistical inference for its

measure of synergy. So while it quantifies synergy, it does not determine whether that quantity is significantly different from a quantity indicating additivity. While those are the most important drawbacks of the Median Effect method, Greco et al identified a number of other drawbacks, including overestimating antagonism at low responses, due to nonlinearity in the median effect plot [11].

The Greco method of identifying synergy [12] is a response surface method similar to the Plummer and Short method described in Section 2.3. Like the Plummer and Short method it has no restrictions on the study design, but it can only identify global synergy between two drugs. Snyder et al have extended the Greco model to handle 3 drugs [29]. The extended model is able to identify 3-way synergy based on both Definition I and Definition II in Section 3.1. But, the extended model still assumes any synergy or antagonism is global, and cannot handle regions of local synergy or antagonism.

Another extension of the Greco method has been done by White et al [32, 31]. This extension can also identify 3-way synergy based on both definitions of Section 3.1, and it can identify local synergy or antagonism, not just global synergy or antagonism. But one drawback of this method is that although it can identify portions of a design ray where synergy is statistically significant, it cannot identify regions where synergy is statistically significant. Another drawback to this method is that it is a complex, multi-step method that requires careful model building at a number of steps. In the cited study of 3 drugs [32], the first analysis stage produced 40 polynomial models, while the second stage reduced that to 10 hierarchical models, and the final stage produced a single hierarchical model. None of the 2 drug response surface methods described above required that level of modeling complexity. The Kong and Lee parametric method uses a relatively simple backwards elimination procedure to select its model, while the Plummer and Short method considers only a single model. The thin-plate splines of the Kong and Lee semiparametric provide flexible polynomial models that require no selection of models or knots.

Chapter 3

Defining Synergy with Three or More Drugs

3.1 Informal Definitions

An informal definition of drug synergy for two drugs is that two drugs are synergistic if their effect when used together is greater than would be expected, based on their effects when used alone. A slightly less informal definition of synergy for two drugs is that two drugs are synergistic (or antagonistic) if there is a departure from additivity.

If a third drug is included, then the informal definition can be easily extended: three drugs are synergistic if their effect when used together is greater than would be expected, based on their effects when used alone. This definition of synergy between three drugs will be referred to as "Definition I" of three drug synergy.

But this informal definition of three drug synergy does not distinguish some important differences in cases of synergy. With three drugs, it is possible that there is synergy between only a pair of drugs, such that the pair of drugs is synergistic regardless of whether or not the third drug is present. This type of synergy will be referred to as "2-way synergy". It is also possible that there is synergy that truly depends on all three drugs, and only exists when all three drugs are present. This type of synergy will be referred to as "3-way synergy".

An informal definition of three drug synergy that does distinguish between 2-way synergy and 3-way synergy is that three drugs are synergistic if their effect when used together is greater than would be expected, based on their effects when used alone, and based on their effects when used in pair-wise combinations, taking into account any 2-way synergy. This definition of synergy between three drugs will be referred to as "Definition II".

Returning to the slightly less informal definition of synergy as a departure from additivity, that definition cannot distinguish between all types of synergy when three drugs are involved. It corresponds to synergy as defined by "Definition I", but does not correspond to synergy as defined by "Definition II".

Both informal definitions of synergy can be useful in different applications. In cancer treatment, where drugs may have severe side effects, it can be important to distinguish between 2-way synergy and 3-way synergy, so a third drug is only included in the treatment if it is truly necessary for the synergistic relationship. In this case Definition II would be preferred. Definition II would also be preferred if previous studies have shown that there is 2-way synergy between one or more pairs of the drugs, in which case it would be important to distinguish between the synergy already expected from the two drugs and any additional synergy. But, if there is no additional cost or penalty if an extraneous drug is included in the treatment, or if previous studies or prior knowledge have shown that there is no 2-way synergy between any pair of the 3 drugs, then the simpler Definition I could be used.

3.2 Graphical Illustration

Two drug synergy is frequently illustrated using "isobolograms". In an isobologram, lines connect drug doses with equal responses. If the two drugs are additive and the drugs have constant relative potency, then the lines connecting equivalent doses will all be straight, diagonal lines. If the individual dose response curves are monotonic, and higher doses have "better" responses (where better may be higher in some models and lower in others), then any departure from additivity can be identified by the curvature of the lines connecting the doses with equal responses. If synergism is present, then the lines will be curved "inward", towards the origin, reflecting how the same response is achieved with lower doses than would be expected if the drugs were additive. Similarly, if antagonism is present, the lines will be curved "outward", away from the origin. As long as the monotonicity and "better" assumptions are met, the interpretation of the direction of the curvature is the same regardless of whether a higher response is better or a lower response is better.

The three panels in Figure 3.1 show a single isobologram illustrating additivity, synergy, and antagonism. For simplicity, the two drugs are assumed to have equal potency. In each panel, the isobologram passes through the dose combination of 10 units of drug 1, with no drug 2. In the additive panel, if that dose of drug 1 is reduced from 10 units to 5 units, it is necessary to increase the dose of drug 2 to 5 units in order to maintain the same response level. But, in the synergy panel, if the dose of drug 1 is reduced to 5 units, it is only necessary to increase the dose of drug 2 to approximately 2 units to maintain the same response level. While in the antagonism panel, if the dose of drug 1 is reduced to 5 units, it is necessary to increase the dose of drug 2 to approximately 8 units to maintain the same response level.

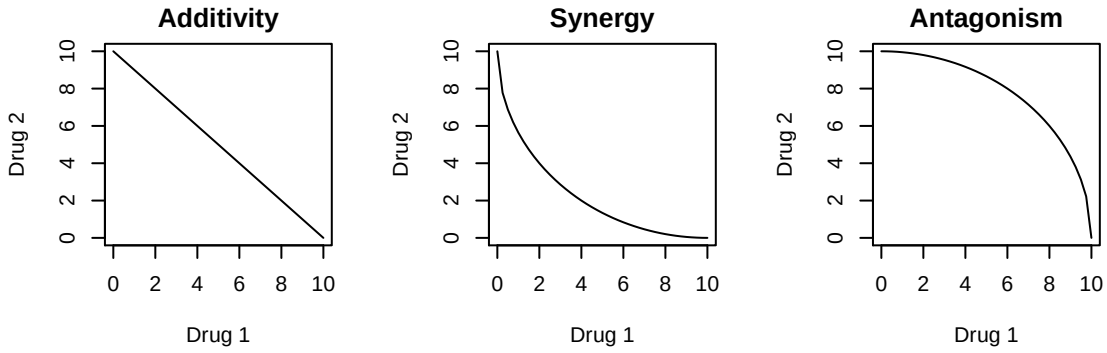

Figure 3.1: Two-drug isobolograms showing additivity, synergy and antagonism.

Isobolograms for two drug synergy are projections of a 2-dimensional figure in 3-dimensional space, onto a graph in 2-dimensional space. The three dimensions are the doses of the two drugs, and their response. Adding a third drug turns the isobolograms into 3-dimensional figures in 4-dimensional space, which cannot be easily visualized. While isobolograms were lines in 2-dimensional space for the two drug case, they now become surfaces in 3-dimensional space for the three drug case. Multiple lines, representing multiple response levels in the two drug case, could easily be shown on a single graph. Because it is difficult to show multiple surfaces on a single graph, as required for the three drug case, this paper will generally show three-drug isobolograms using multiple graphs, with separate graphs for different dose levels of one of the drugs.

As described above, an additive response in the two drug case appears as a straight, diagonal line in an isobologram for two drugs. For the three drug case, an additive response appears as a flat surface, as shown in Figure 3.2.

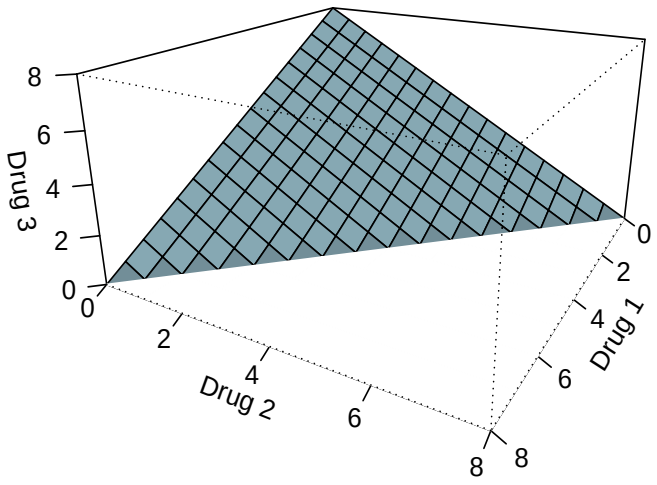

Figure 3.2: Additivity between all three drugs.

A particular combination treatment is represented by a point on an isobologram. If the combination treatment is additive, then the point will be on the surface in Figure 3.2. If the point is below the surface, but has the same response as the surface, then that combination treatment is synergistic. If the point is above the surface, but has the same response as the surface, then that combination treatment is antagonistic.

As described in the previous section, with three drugs there can be multiple definitions of synergy (and antagonism), and isobolograms can be useful to help visualize the different definitions.

Figure 3.3 shows an isobologram surface for a response where there is 2-way synergy between drugs 1 and 3, but additivity between drugs 1 and 2 and between drugs 2 and 3, and no 3-way synergy between all three drugs. The surface is no longer flat, reflecting the synergy between drugs 1 and 3. Although this is only 2-way synergy and drug 3 plays no role in the synergy, a point on this surface would be identified as synergy by Definition I of three drug synergy, because the point is below the surface in Figure 3.2.

In order to meet the requirements of Definition II of three drug synergy, a point would have to be below the surface in Figure 3.3, not just below the surface in Figure 3.2.

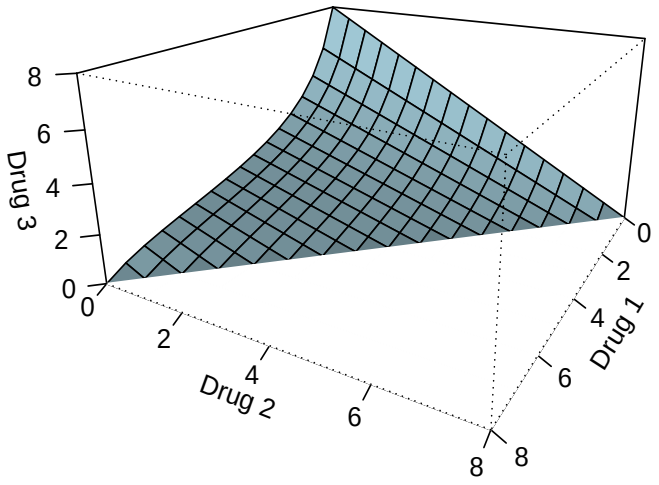

Figure 3.3: Synergy between drugs 1 and 3, Additivity between drugs 1 and 2 and between drugs 2 and 3.

Figure 3.4 shows an isobologram surface for a response where there is 2-way synergy between all three pairs of drugs, but still no 3-way synergy. The surface is curved, reflecting the 2-way synergy between the pairs of drugs. As in Figure 3.3, a point on this surface would be identified as synergy by Definition I of three drug synergy, because the point is below the surface in Figure 3.2. In order to meet the requirements of Definition II of three drug synergy, a point would have to be below the surface in Figure 3.4, not just below the surface in Figure 3.2.

Figure 3.5 shows an isobologram surface for a response where there is 2-way synergy between all three pairs of drugs, and 3-way synergy between the three drugs. A point on this surface would be identified as synergy by both Definition I and Definition II of three drug synergy.

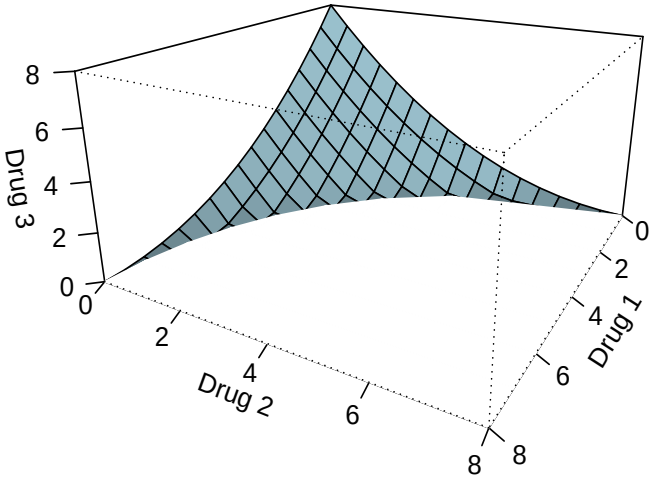

Figure 3.4: 2-Way synergy between all three pairs of drugs.

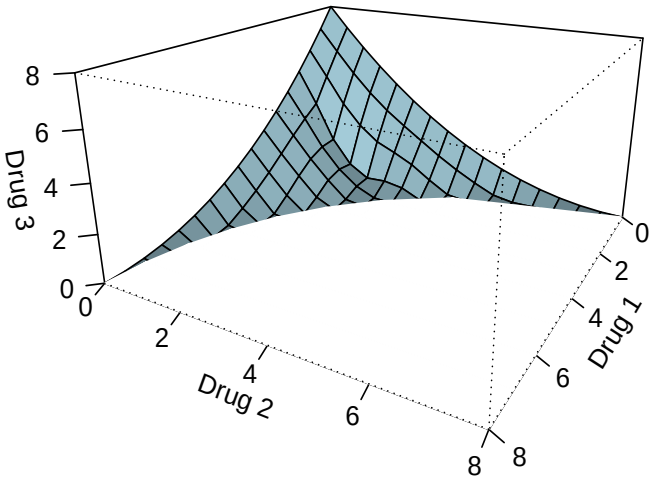

Figure 3.5: 2-Way synergy between all three pairs of drugs and 3-Way synergy between the three drugs.

3.3 Formal Definitions

A number of formal definitions have been proposed for drug synergy [24] [4] [16]. The Bliss independence model and the Loewe additivity model are the two most cited models [21]. The Loewe additivity model is more broadly applicable (the Bliss model is limited to outcomes that are proportions), and is consistent with the graphical isobologram approach described above, so it will be the formal model used in this thesis.

For 2 drugs, the traditional Loewe additivity model can be defined as:

$$\frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}} = II \begin{cases} < 1 & \text{Synergism,} \\ = 1 & \text{Additivity,} \\ > 1 & \text{Antagonism} \end{cases} \tag{3.1}$$

where d_1 is the dose of drug 1 in the combination of two drugs and d_2 is the dose of drug 2 in the combination. Assuming that y is the effect of the combination of the two drugs at the given doses (d_1, d_2), $D_{y,1}$ is the dose of drug 1 that would be required to produce the same effect y if drug 1 had been used alone, while $D_{y,2}$ is the dose of drug 2 that would be required to produce the same effect y if drug 2 had been used alone. II is the "interaction index", and represents one of the three possible outcomes shown above [21].

To accommodate 3 drugs, a simple extension of this model has been used [3]:

$$\frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}} + \frac{d_3}{D_{y,3}} = II \begin{cases} < 1 & \text{Synergism,} \\ = 1 & \text{Additivity,} \\ > 1 & \text{Antagonism} \end{cases} \tag{3.2}$$

In this extension, y is the effect of the combination of the three drugs at the given doses (d_1, d_2, d_3), d_3 is the dose of drug 3 in the combination treatment, $D_{y,3}$ is the dose of drug 3 that would be required to produce the same effect y if drug 3 had been used alone, and d_1, d_2, $D_{y,1}$, $D_{y,2}$ and II have the same meaning as in (3.1).

It is possible to show that the 3 drug interaction index has the properties of an interaction index, namely that $II = 1$ for additivity, that $II < 1$ for synergy, and that $II > 1$ for antagonism.

$$II = \frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}} + \frac{d_3}{D_{y,3}}$$

$$II = \frac{D_{y,1}}{D_{y,1}} \left(\frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}} + \frac{d_3}{D_{y,3}} \right)$$

$$II = \frac{1}{D_{y,1}} \left(d_1 + d_2 \frac{D_{y,1}}{D_{y,2}} + d_3 \frac{D_{y,1}}{D_{y,3}} \right)$$

$$II = \frac{1}{D_{y,1}} \left(d_1 + \rho_2 d_2 + \rho_3 d_3 \right)$$

where ρ_2 is the relative potency of Drug 2 to Drug 1, and ρ_3 is the relative potency of Drug 3 to Drug 1.

If there is additivity between the 3 drugs, then $d_1 + \rho_2 d_2 + \rho_3 d_3 = D_{y,1}$ and $II = 1$. If there is synergy between the 3 drugs, then $d_1 + \rho_2 d_2 + \rho_3 d_3 < D_{y,1}$ and $II < 1$. If there is antagonism between the 3 drugs, then $d_1 + \rho_2 d_2 + \rho_3 d_3 > D_{y,1}$ and $II > 1$. So (3.2) has all of the properties of an interaction index.

To accommodate n drugs, a further extension of the Loewe additivity model can been specified as [3]:

$$\sum_{j=1}^{n} \frac{d_j}{D_{y,j}} = II \begin{cases} < 1 & \text{Synergism,} \\ = 1 & \text{Additivity,} \\ > 1 & \text{Antagonism} \end{cases} \tag{3.3}$$

In this extension, y is the effect of the combination of the n drugs at the given doses $(d_1, \ldots, d_n)$. d_j, where $1 \leq j \leq n$, is the dose of drug j in the combination treatment, and $D_{y,j}$ is the dose of drug j that would be required to produce the same effect y if drug j had been used alone. II has the same interpretation as in (3.1) and (3.2). In this extension, II can be shown to have the properties of an interaction index using an argument similar to that used above for the 3-drug interaction index (3.2).

The models above correspond to the informal "Definition I" of synergy. Although we have done considerable work attempting to develop a formal model that corresponds

to the informal "Definition II" of synergy, a successful model has not yet been found. Such a model will remain open as an area for future research.

Chapter 4

Extend Plummer and Short's Model to Three or More Drugs

4.1 General Model Description

The first synergy method that this thesis extends to handle more than two drugs is Plummer and Short's method, which was described in Section 2.3.

Assume there are n drugs, where each drug is uniquely identified by a positive integer j, $1 \leq j \leq n$.

Assume the individual drugs have straight log dose-response curves, although the curves need not be parallel. The response for drug 1 can be specified as:

$$Y = \beta_0 + \beta_1 \log(d_1) \tag{4.1}$$

where Y is the response, d_1 is the dose of Drug 1, and β_0 and β_1 are respectively the intercept and slope of the linear log dose-response curve.

As in Plummer and Short's method, given that the log-dose response curve of drug j is also linear, the logarithm of the relative potency of drug 1 and drug j is linearly related to the log dose of either drug, so the relative potency of drug 1 and drug j can be expressed as:

$$\log(\rho_j) = \beta_{2j-2} + \beta_{2j-1} \log(d_j)$$

where ρ_j is the relative potency, and β_{2j-2} and β_{2j-1} are constants.

When both drug 1 and drug j are present, we can treat the combination of the two drugs as a dose D_j of drug j alone, in which some amount of drug j has been replaced by an amount of drug 1 with an equivalent effect:

$$D_j = d_j + d_1/\rho_j$$

The relative potency at this level is then:

$$\log(\rho_j) = \beta_{2j-2} + \beta_{2j-1}\log(D_j) \tag{4.2}$$

While each drug j, $2 \leq j \leq n$, has its relative potency compared to drug 1, ρ_j, defined as above, for notational convenience we will also define $\rho_1 = 1$ (which could be interpreted as the relative potency of drug 1 to itself).

With n drugs there can be a number of different kinds of drug interactions, which will be characterized by the number of drugs involved in the interaction, and referred to as "2-way interactions", "3-way interactions", etc., all the way up to an "n-way interaction". The "2-way interactions" are between a single pair of drugs, and do not depend in any way on the presence or absence of any of the other drugs. Similarly, "3-way interactions" are between three of the n drugs, and only depend on the presence of the three drugs involved in the interaction. The "n-way interaction" is an interaction between all n drugs, and depends on the presence of all n drugs.

With n drugs, for each of the "p-way interactions", $2 \leq p \leq n$, there are $m_p = \binom{n}{p}$ different interactions, each of which is between a unique combination of p of the n drugs, and a total of $\sum_{p=2}^{n} m_p$ interactions.

An extended Plummer and Short model for n drugs can be defined as:

$$Y = \beta_0 + \beta_1 \log\left(\sum_{j=1}^{n} \rho_j d_j + \sum_{p=2}^{n} \sum_{1 \leq i_1 < i_2 < \cdots < i_p \leq n} \gamma_{i_1 i_2 \ldots i_p} \left(\prod_{j=1}^{p} \rho_{i_j} d_{i_j}\right)^{1/p}\right)$$

where the relative potency parameters ρ_j are defined as above and d_j is the amount of Drug j in the combination. In addition, i_1, i_2, …, i_p, where $1 \leq i_1 < i_2 < \ldots i_p \leq n$, are the drugs involved in a p-way interaction, with respective doses d_{i_j} and respective relative potencies to Drug 1 of ρ_{i_j}.

The monotonicity assumption (4.1) implies that a larger dose of a drug has a "better" effect, although better could mean either larger or smaller; when a larger effect is better, $\beta_1 > 0$, and when a smaller effect is better, $\beta_1 < 0$.

The γ coefficients model the interactions between combinations of drugs, with the coefficient $\gamma_{i_1 i_2 \ldots i_p}$ modeling the p-way interaction between drugs i_1, i_2, …, i_p. For

all of the γ parameters in the model, positive values indicate synergy, negative values indicate antagonism, and a value of zero indicates additivity.

4.2 Three Drug Model Description

We assume there are three drugs, 1, 2, and 3. An extended Plummer and Short model can then be defined as:

$$\begin{aligned} Y = \beta_0 + \beta_1 \log(d_1 + \rho_2 d_2 + \rho_3 d_3 \\ + \gamma_{12} (d_1 \rho_2 d_2)^{1/2} + \gamma_{13} (d_1 \rho_3 d_3)^{1/2} + \gamma_{23} (\rho_2 d_2 \rho_3 d_3)^{1/2} \\ + \gamma_{123} (d_1 \rho_2 d_2 \rho_3 d_3)^{1/3}) \end{aligned} \tag{4.3}$$

where ρ_2 and ρ_3 are defined as in (4.2), and D_2 and D_3 are respectively the solutions to:

$$D_2 = d_2 + d_1 e^{-\beta_2 - \beta_3 \log(D_2)} \quad (= d_2 + d_1/\rho_2) \tag{4.4}$$

$$D_3 = d_3 + d_1 e^{-\beta_4 - \beta_5 \log(D_3)} \quad (= d_3 + d_1/\rho_3) \tag{4.5}$$

The model can assess 3-way drug synergy as defined by Definition II of synergy in Section 3.1 (synergy as defined by Definition I could be assessed by removing the model terms containing γ_{12}, γ_{13}, and γ_{13}).

In the model, β_0 and β_1 represent the intercept and slope of the log dose-response curve for drug 1 alone. β_2 and β_3 describe the difference between the drug 2 and drug 1 log dose-response curves, with β_2 corresponding to the "horizontal" difference between the lines and β_3 corresponding to the difference of slopes. If the log dose-response curves are parallel, then $\beta_3 = 0$. β_4 and β_5 describe the difference between the drug 3 and drug 1 log dose-response curves, with β_4 corresponding to the "horizontal" difference between the lines and β_5 corresponding to the difference of slopes. If the log dose-response curves are parallel, then $\beta_5 = 0$.

The rest of the coefficients in the model describe the synergy relationships. γ_{12} is a coefficient of synergy for 2-way synergy between drugs 1 and drug 2, γ_{13} is a coefficient of synergy for 2-way synergy between drugs 1 and 3, γ_{23} is a coefficient of synergy for 2-way synergy between drugs 2 and 3, and γ_{123} is a coefficient of synergy

for 3-way synergy between all 3 drugs. For all of the synergy parameters in the model, positive values indicate synergy, negative values indicate antagonism, and a value of zero indicates additivity.

The model can be fitted using iterative methods for nonlinear regression, solving (4.4) and (4.5) numerically at each iteration for each dose combination to determine D_2 and D_3. The presence of synergy can be tested by fitting full and reduced models with and without the γ_{12}, γ_{13}, γ_{23} and γ_{123} terms, or by testing the significance of each of those terms in the full model.

It is possible to show the equivalence of (4.3) and the Interaction Index of the 3-drug Loewe additivity model in (3.2); the steps follow those that Lee et al [21] used to show the equivalence of the Plummer and Short model and the 2-drug Loewe additivity model.

In (4.3), if $d_2 = d_3 = 0$, then $Y = \beta_0 + \beta_1 \log(d_1)$, and $\exp\left(\frac{Y-\beta_0}{\beta_1}\right) = D_{y,1}$ (recall from Section 3.3 that $D_{y,1}$ is the dose of drug 1 that would have to be used alone to have the same effect y). Similarly, if $d_1 = d_3 = 0$, then $Y = \beta_0 + \beta_1 \log(\rho_2 d_2)$, and $\rho_2^{-1} \exp\left(\frac{Y-\beta_0}{\beta_1}\right) = D_{y,2}$, while if $d_1 = d_2 = 0$, then $Y = \beta_0 + \beta_1 \log(\rho_3 d_3)$, and $\rho_3^{-1} \exp\left(\frac{Y-\beta_0}{\beta_1}\right) = D_{y,3}$.

Next, for notational simplicity, let

$$\Lambda = \gamma_{12}(d_1\rho_2 d_2)^{1/2} + \gamma_{13}(d_1\rho_3 d_3)^{1/2} + \gamma_{23}(\rho_2 d_2 \rho_3 d_3)^{1/2} + \gamma_{123}(d_1\rho_2 d_2 \rho_3 d_3)^{1/3}$$

so (4.3) can be rewritten more compactly as

$$Y = \beta_0 + \beta_1 \log(d_1 + \rho_2 d_2 + \rho_3 d_3 + \Lambda)$$

The following steps can then be used to show the equivalence of (4.3) and the

Interaction Index of the 3-drug Loewe Additivity Model (3.2).

$$\begin{aligned}
\exp\left(\frac{Y-\beta_0}{\beta_1}\right) &= d_1 + \rho_2 d_2 + \rho_3 d_3 + \Lambda \\
\exp\left(\frac{Y-\beta_0}{\beta_1}\right) - \Lambda &= d_1 + \rho_2 d_2 + \rho_3 d_3 \\
1 - \frac{\Lambda}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} &= \frac{d_1}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} + \frac{\rho_2 d_2}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} + \frac{\rho_3 d_3}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} \\
1 - \frac{\Lambda}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} &= \frac{d_1}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} + \frac{d_2}{\rho_2^{-1}\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} + \frac{d_3}{\rho_3^{-1}\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} \\
1 - \frac{\Lambda}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} &= \frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}} + \frac{d_3}{D_{y,3}}
\end{aligned}$$

So, returning to the original notation, the Interaction Index of the Loewe additivity model is equivalent to

$$II = 1 - \frac{\gamma_{12}\left(d_1\rho_2 d_2\right)^{1/2} + \gamma_{13}\left(d_1\rho_3 d_3\right)^{1/2} + \gamma_{23}\left(\rho_2 d_2 \rho_3 d_3\right)^{1/2} + \gamma_{123}\left(d_1\rho_2 d_2\rho_3 d_3\right)^{1/3}}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)}$$

4.3 Response Surface Estimation

An R program was used to implement the extended Plummer and Short method described in the previous section.

Five data sets were constructed and analyzed using the program. The five constructed data sets corresponded to 5 different synergy scenarios: additivity between all 3 drugs, 2-way synergy between one pair of the drugs, 3-way synergy between all of the drugs, 2-way synergy between one pair of drugs combined with 3-way synergy between all of the drugs, and a combination of localized 3-way synergy and 3-way antagonism (this last scenario violates the model's assumption of global synergy so the model is not expected to perform well).

Each constructed data set consisted of generated measurements for combination treatments of 3 drugs. All 3 drugs had 7 dose levels. In addition to a dose of 0, the first drug had dose levels of 2.5, 5, 7.5, 10, 15, and 20. The second drug had levels one half of the first drug, while the third had levels one quarter of the first drug. The relative potency of the first drug to the second drug was defined as ρ_2:

$$\log\left(\rho_2\right) = \beta_2 + \beta_3 \log\left(D_2\right) \tag{4.6}$$

where D_2 is the solution to:

$$D_2 = d_2 + d_1/\rho_2$$

and $\beta_2 = \log 2$ and $\beta_3 = 0.02$. The relative potency of the first drug to the third drug was defined as ρ_3:

$$\log(\rho_3) = \beta_4 + \beta_5 \log(D_3) \tag{4.7}$$

where D_3 is the solution to:

$$D_3 = d_3 + d_1/\rho_3$$

and $\beta_4 = \log 3$ and $\beta_5 = 0.005$. All possible combinations of the 3 drugs at the given dose levels were used, with three generated results (repetitions) at each combination.

The generated response variable was a proportion, such as the proportion of cells viable after the given treatment, a common end point in many nonclinical oncology studies. Because the analysis program assumes the response E is a proportion, it initially transforms it to be the transformed effect Y using the function $g(E) = \text{logit}\, E = \log \frac{E}{1-E}$. All generated responses were constructed on the transformed scale as y, and then transformed to be E on the original scale of responses using the function $g^{-1}(y) = \text{logit}^{-1} y = \frac{1}{1+\exp(-y)}$. A small amount of random noise, from a normal distribution with a mean of 0 and a standard deviation of 0.1, was added to each generated response value before its antilogit transformation.

4.3.1 First Scenario: Additivity

The constructed data set for the first scenario assumed all 3 drugs were additive. The response was constructed as:

$$y = \beta_0 + \beta_1 \log(d_1 + \rho_2 d_2 + \rho_3 d_3) + \epsilon$$

where

$$\epsilon \sim N\left(0, 0.1^2\right)$$

and where $\beta_0 = 4$, $\beta_1 = -1$, and ρ_2 and ρ_3 were defined as in (4.6) and (4.7) respectively, with $\beta_2 = \log 2$, $\beta_3 = 0.02$, $\beta_4 = \log 3$, and $\beta_5 = 0.005$.

The analysis of the first scenario found no significant two-way interactions (p = 0.149, p = 0.060, and p = 0.392) and no significant three-way interaction (p = 0.122).

Figure 4.1 shows the results of the analysis. Panels A through C of the figure show the fitted log dose-response curves for each individual drug, used alone. The log-dose response curves were used to construct initial estimates for the extended Plummer and Short model.

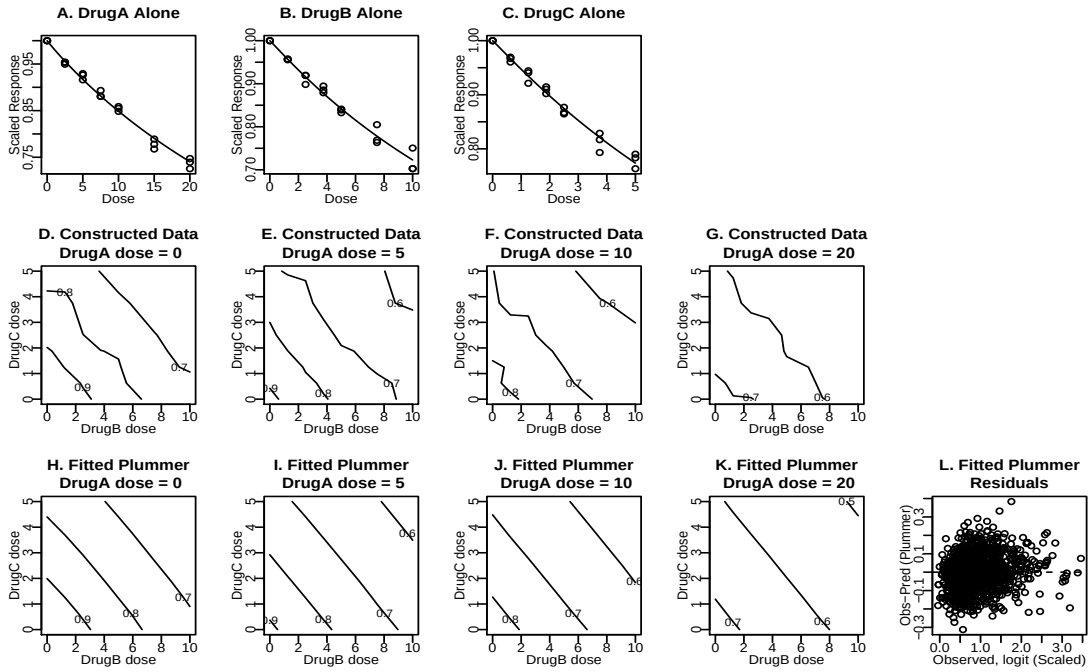

Figure 4.1: Results from first scenario, with all additive relations.

Panels D through G in the figure show contour plots of the "observed" constructed data, with each panel showing the contour plot at a different dose of Drug A. Each plot shows the response contours for the given doses of Drug B and Drug C, on the X and Y axes respectively. The generally straight contour lines in all four panels, i.e. at all doses of Drug A, reflect the additivity between the drugs.

Panels H through K in the figure show contour plots of the fitted model. The plots closely match the corresponding plots in panels D through G, showing that the model has accurately fitted the "observed" data. Panel L shows the residuals from the fitted model, plotted against the observed (logit) response.

4.3.2 Second Scenario: Two-Way Synergy

The constructed data set for the second scenario assumed that there was 2-way synergy between drugs 2 and 3, but all other relations were additive. The response was constructed as:

$$y = \beta_0 + \beta_1 \log\left(d_1 + \rho_2 d_2 + \rho_3 d_3 + \gamma_{23}\left(\rho_2 d_2 \rho_3 d_3\right)^{\frac{1}{2}}\right) + \epsilon$$

where

$$\epsilon \sim N\left(0, 0.1^2\right)$$

and where $\beta_0 = 4$, $\beta_1 = -1$, ρ_2 and ρ_3 were defined as in (4.6) and (4.7) respectively, with $\beta_2 = \log 2$, $\beta_3 = 0.02$, $\beta_4 = \log 3$, $\beta_5 = 0.005$, and $\gamma_{23} = 2$.

The analysis of the second scenario found a significant interaction between the second and third drugs ($p < 0.001$), although neither of the other two-way interactions were significant ($p = 0.858$ and $p = 0.291$), nor was the three-way interaction ($p = 0.605$). The coefficient for the interaction between the second and third drugs, γ_{23}, was estimated to be 1.91 (95% confidence interval: (1.68, 2.17)), with its positive sign indicating that this interaction is synergistic, not antagonistic.

Figure 4.2 shows the results for the second constructed data set. As in the previous scenario, the log-dose response curves in Panels A through C were used to construct initial estimates for the extended Plummer and Short model.

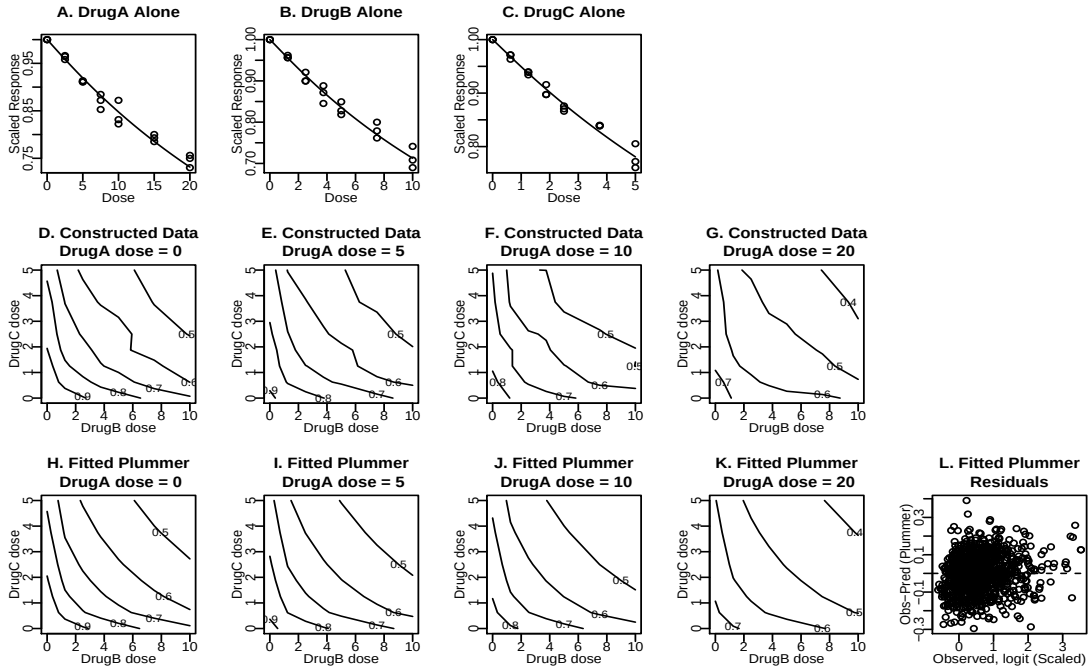

Figure 4.2: Results from second scenario, with 2-way synergy between Drug B and Drug C, but additivity for all other relations.

In this scenario, the contour plots of the "observed" data in Panels D through G are now curved, reflecting the 2-way synergy between Drug B and Drug C.

Panels H through K show contour plots of the fitted model, and again the plots closely match the corresponding plots in panels D through G, showing that the model has accurately fitted the "observed" data. As before, Panel L shows the residuals from the fitted model, plotted against the observed (logit) response.

4.3.3 Third Scenario: Three-Way Synergy

The constructed data set for the third scenario assumed that there was 3-way synergy between all 3 drugs, but all 2-way relations were additive. The response was constructed as:

$$y = \beta_0 + \beta_1 \log\left(d_1 + \rho_2 d_2 + \rho_3 d_3 + \gamma_{123}\left(d_1 \rho_2 d_2 \rho_3 d_3\right)^{\frac{1}{3}}\right) + \epsilon$$

where

$$\epsilon \sim N\left(0, 0.1^2\right)$$

and where $\beta_0 = 4$, $\beta_1 = -1$, ρ_2 and ρ_3 were defined as in (4.6) and (4.7) respectively, with $\beta_2 = \log 2$, $\beta_3 = 0.02$, $\beta_4 = \log 3$, $\beta_5 = 0.005$, and $\gamma_{123} = 1.5$.

The analysis of the third scenario found a significant three-way interaction between the drugs ($p < 0.001$), although none of the two-way interactions were significant ($p = 0.251$, $p = 0.103$ and $p = 0.231$). The coefficient for the three-way interaction between the drugs, γ_{123}, was estimated to be 1.57 (95% confidence interval: (1.45, 1.68)), with its positive sign indicating that this interaction is synergistic, not antagonistic.

Figure 4.3 shows the results for the third constructed data set. As in the previous scenarios, the log-dose response curves in Panels A through C were used to construct initial estimates for the extended Plummer and Short model.

Also as in the previous scenario, the contour plots of the "observed" data in Panels D through G are still curved, reflecting the 3-way synergy between Drug A, Drug B and Drug C. But the lines in Panel D are straight, reflecting the fact that this is 3-way synergy that depends on Drug A being present. In the previous scenario, the lines in Panel D were curved, because the 2-way synergy between Drug B and Drug C was

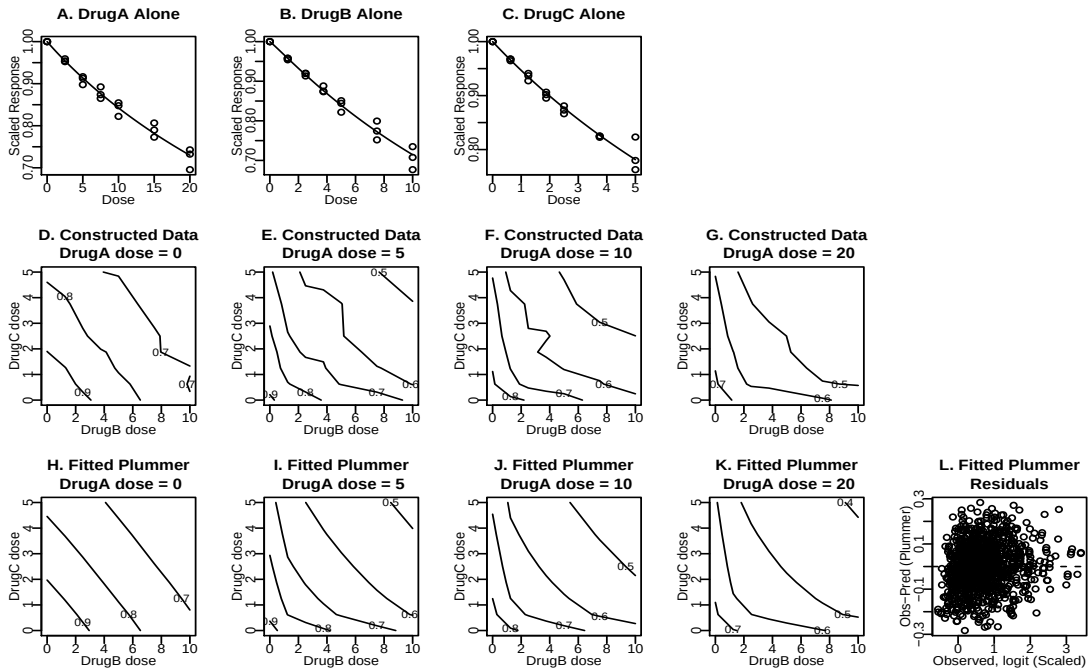

Figure 4.3: Results from third scenario, with 3-way synergy between Drug A, Drug B and Drug C, but additivity between each pair of drugs.

present even in the absence of Drug A.

Panels H through K show contour plots of the fitted model, and once again the plots closely match the corresponding plots in panels D through G, showing that the model has accurately fitted the "observed" data. As before, Panel L shows the residuals from the fitted model, plotted against the observed (logit) response.

4.3.4 Fourth Scenario: Two-Way Synergy and Three-Way Synergy

The constructed data set for the fourth scenario assumed that there was 2-way synergy between one pair of drugs and 3-way synergy between all 3 drugs. The response was constructed as:

$$y = \beta_0 + \beta_1 \log\left(d_1 + \rho_2 d_2 + \rho_3 d_3 + \gamma_{23}\left(\rho_2 d_2 \rho_3 d_3\right)^{\frac{1}{2}} + \gamma_{123}\left(d_1 \rho_2 d_2 \rho_3 d_3\right)^{\frac{1}{3}}\right) + \epsilon$$

where

$$\epsilon \sim N\left(0, 0.1^2\right)$$

and where $\beta_0 = 4$, $\beta_1 = -1$, ρ_2 and ρ_3 were defined as in (4.6) and (4.7) respectively, with $\beta_2 = \log 2$, $\beta_3 = 0.02$, $\beta_4 = \log 3$, $\beta_5 = 0.005$, $\gamma_{23} = 2$, and $\gamma_{123} = 1.5$.

The analysis of the fourth scenario found a significant interaction between the second and third drugs ($p < 0.001$) and a significant three-way interaction between all three drugs ($p < 0.001$), but the other two-way interactions were not significant ($p = 0.071$ and $p = 0.163$). The coefficient for the interaction between the second and third drugs, γ_{23}, was estimated to be 2.08 (95% confidence interval: (1.82, 2.37)), while the coefficient for the three-way interaction between all three drugs, γ_{123}, was estimated to be 1.45 (95% confidence interval: (1.31, 1.60)), with both positive signs indicating that the interactions are synergistic, not antagonistic.

Figure 4.4 shows the results for the fourth constructed data set. As in the previous scenarios, the log-dose response curves in Panels A through C were used to construct initial estimates for the extended Plummer and Short model.

As in both previous scenarios, the contour plots of the "observed" data in Panels E through G are still curved, reflecting the 2-way synergy between Drug A and Drug

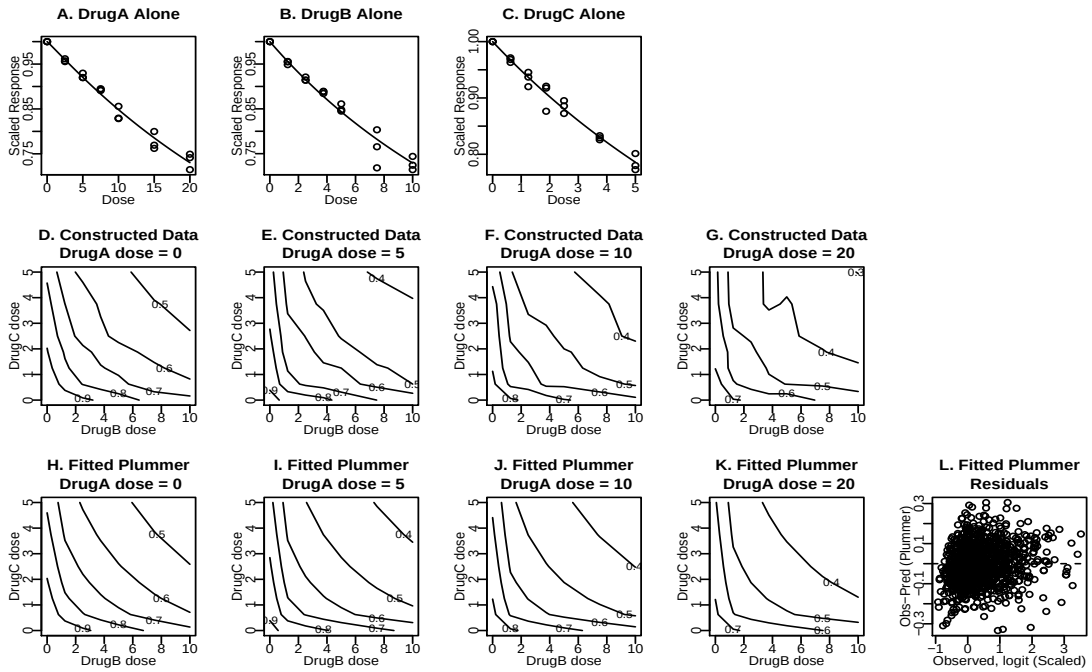

Figure 4.4: Results from fourth scenario, with 2-way synergy between Drug A and Drug B, and 3-way synergy between Drug A, Drug B and Drug C.

B, and the 3-way synergy between Drug A, Drug B and Drug C. Unlike the previous scenario (but like the second scenario), the lines in Panel D are curved, because the 2-way synergy between Drug B and Drug C is still present even in the absence of Drug A.

Panels H through K show contour plots of the fitted model, and once again the plots closely match the corresponding plots in panels D through G, showing that the model has accurately fitted the "observed" data. As before, Panel L shows the residuals from the fitted model, plotted against the observed (logit) response.

4.3.5 Fifth Scenario: Local Three-Way Synergy and Antagonism

The constructed data set for the fifth scenario assumed that there was local 3-way synergy between all 3 drugs in one region, but local 3-way antagonism between all 3 drugs in another region; all 2-way relations were assumed to be additive. The response was constructed as:

$$\begin{aligned} y = \beta_0 + \beta_1 \log\big(& d_1 + \rho_2 d_2 + \rho_3 d_3 \\ & + f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right)\left(d_1 \rho_2 d_2 \rho_3 d_3\right)^{\frac{1}{3}} \big) + \epsilon \end{aligned}$$

where:

$$\begin{aligned} f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right) = & \kappa_{123,0} + \kappa_{123,1} d_1^{\frac{1}{3}} + \kappa_{123,2}\left(\rho_2 d_2\right)^{\frac{1}{3}} + \kappa_{123,3}\left(\rho_3 d_3\right)^{\frac{1}{3}} \\ & + \kappa_{123,4} d_1 + \kappa_{123,5} \rho_2 d_2 + \kappa_{123,6} \rho_3 d_3 \\ & + \kappa_{123,7}\left(d_1 \rho_2 d_2 \rho_3 d_3\right)^{\frac{1}{3}} \end{aligned}$$

and

$$\epsilon \sim N\left(0, 0.1^2\right)$$

and $\beta_0 = 4$, $\beta_1 = -1$, ρ_2 and ρ_3 were defined as in (4.6) and (4.7) respectively, with $\beta_2 = \log 2$, $\beta_3 = 0.02$, $\beta_4 = \log 3$, $\beta_5 = 0.005$, $\kappa_{123,0} = 1$, $\kappa_{123,3} = 0.5$, $\kappa_{123,7} = -0.2$, and $\kappa_{123,i} = 0, i \in \{1, 2, 4, 5, 6\}$.

This scenario violates one of the underlying assumptions of the Plummer and Short model, that any synergy or antagonism is "global" and the same at all dose levels, because the interaction depends on the function f_{123}, which depends on the dose levels.

The result of violating this assumption can be seen in the inaccurate estimates that follow.

The analysis of the fifth scenario found all of the two-way interactions to be significant ($p = 0.004$ for γ_{12}, $p = 0.010$ for γ_{13} and γ_{23}), as well as the three-way interaction ($p = 0.020$). The coefficient for the two-way interaction between drugs one and two, γ_{12}, was estimated to be 0.24 (95% confidence interval: (0.08, 0.41)), while the coefficient for the two-way interaction between drugs one and three, γ_{13}, was estimated to be 0.22 (95% confidence interval: (0.05, 0.40)), and the coefficient for the two-way interaction between drugs two and three, γ_{23}, was estimated to be 0.26 (95% confidence interval: (0.08, 0.47)). The coefficient for the three-way interaction between the drugs, γ_{123}, was estimated to be 0.26 (95% confidence interval: (0.03, 0.48)). All four coefficient signs were positive, indicating the interactions are synergistic, not antagonistic.

Figure 4.5 shows the results for the fifth constructed data set. As in the previous scenarios, the log-dose response curves in Panels A through C were used to construct initial estimates for the extended Plummer and Short model.

In this scenario, the contour plots of the "observed" data in Panels D through G, in the region of local 3-way synergy, are curved "down" at and the low dose of Drug A, but not curved consistently in one direction at higher doses of Drug A, reflecting the mix of synergism and antagonism.

As before, Panels H through K show contour plots of the fitted model. In Panels H through K, the lines in the contour plot are consistently curved down, reflecting the (incorrectly) estimated global synergism of the model. Because the model assumes that synergy or antagonism is the same at all dose combinations, and because there were more observations with synergy than with antagonism, the model has determined that synergy is present, even in the regions of local antagonism.

Panel L shows the residuals from the fitted model, plotted against the observed (logit) response. Because the model does not fit the data very well in this scenario, the residuals are much larger than in the previous scenarios.

The large residuals in Panel L and the poor fit in Panel K indicate that a model assumption has been violated, and that the estimated coefficients are not meaningful.

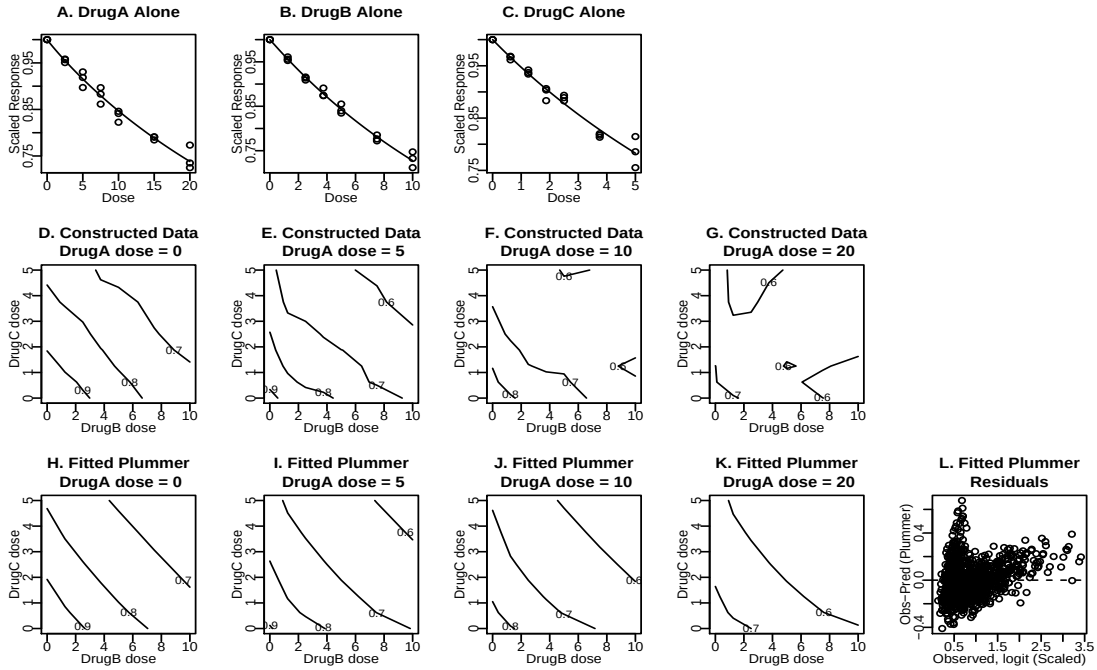

Figure 4.5: Results from fifth scenario, with local 3-way synergy in one region and local 3-way antagonism in another.

4.4 Evaluating Goodness of Fit

Simulated data was created to evaluate the goodness of fit of the extended Plummer and Short method. The simulated data was constructed using the extended Plummer and Short model in (4.3), with known "true" values for the parameters and an additional random component. The random component was taken from a normal distribution with a mean of 0 and a standard deviation of 0.1. The simulated data used the same dose levels as in the original constructed data sets.

The goodness of fit was evaluated for five different scenarios. The first four scenarios corresponded to the first four scenarios evaluated in in the Response Surface Estimation section, 4.3: additivity between all 3 drugs, 2-way synergy between one pair of the drugs, 3-way synergy between all of the drugs, and 2-way synergy between one pair of drugs combined with 3-way synergy between all of the drugs.

The fifth scenario of the Response Surface Estimation section was a scenario with local synergy and antagonism, which violated one of the model assumptions of the extended Plummer and Short method and was not fit well. Rather than evaluating the poorness of the fit for that scenario, the goodness of fit was evaluated in a new scenario. The new scenario had 2-way synergy between one pair of drugs, 2-way antagonism between another pair of drugs, and 3-way synergy between all 3 drugs.

For each scenario 100 simulated data sets were constructed, and each simulated data set was analyzed using the extended Plummer and Short method. The goodness of fit under each scenario was then evaluated using the estimated parameters from each simulated data set. The estimated parameters were used to calculate the mean estimate of each parameter, the bias of each parameter estimate (the mean of the difference of each estimate and the known, true value), the percent bias of each parameter estimate (the bias divided by the true value, times 100%), and the mean standard error of each parameter estimate.

Additionally, the goodness of fit of the model was evaluated based on the classification of each drug interaction as either synergistic, antagonistic or additive. The

classification of an interaction is determined by the statistical significance of its corresponding γ parameter. If the parameter is positive and significant, then the interaction is classified as synergistic; if the parameter is negative and significant, then the interaction is classified as antagonistic; if the parameter is not-significant then the interaction is classified as additive. To evaluate the goodness of fit, for each γ parameter the number of simulation runs that were classified into each category was counted, and the percentage of runs that chose the "correct" category was recorded.

Because the classification is based on the statistical significance of a parameter, the "correct" category for an interaction is not simply based on the true value of the γ parameter for that interaction. Although positive values indicate synergy, positive values close to zero may not be far enough from zero to achieve significant synergy. Similarly, although negative values indicate antagonism, negative values close to zero may not be far enough from zero to achieve significant antagonism. So the "correct" category must take into account not just the true value of the parameter, but also the "expected" significance of the parameter.

The standard errors for the parameters are estimated as part of the nonlinear regression performed on the model of the extended Plummer and Short parametric method. The nonlinear regression software used in this implementation of the extended Plummer and Short parametric method, the nls() function in R, uses the Gauss-Newton method to estimate the regression parameters and their standard errors. The estimated standard errors are based on the design of the model and the residual mean square error, which estimates the standard deviation.

In the simulations used to evaluate the goodness of fit, the true standard deviation is known, and used to create the simulated data. In the same way that the Gauss-Newton method uses the estimated standard deviation to estimate the standard errors of the estimated parameters in the model, the known true standard deviation can be used to calculate a "true standard error" for each known, true parameter. These can then be used to calculate the expected "true" significance of the parameter, which can then be used to define the "correct" category for a drug interaction.

The "true standard error" for each regression parameter was calculated from the

known true standard deviation and a design matrix based on derivatives of the model specified in Equation (4.3). The derivatives are not shown here, but are similar (although simpler) to those shown in Appendix A for the extended Kong and Lee parametric method.

The "true standard errors" for the regression parameters were then used to calculate the expected significance of the parameter, and the classification of its corresponding drug interaction as either synergistic, antagonistic or additive.

Figures 4.6, 4.7, 4.8, and 4.9 summarizes the bias and classification results respectively for the γ_{12}, γ_{13}, γ_{23}, and γ_{123} parameters under all 5 scenarios. The percent bias is generally less than 5%, and the bias is small. The interactions are generally classified correctly at least 90% of the time. The slightly higher than expected rate of mis-classification may be due to slight differences in the calculations performed by the R nls() function and the derivative-based design matrix used to calculate the "true standard errors".

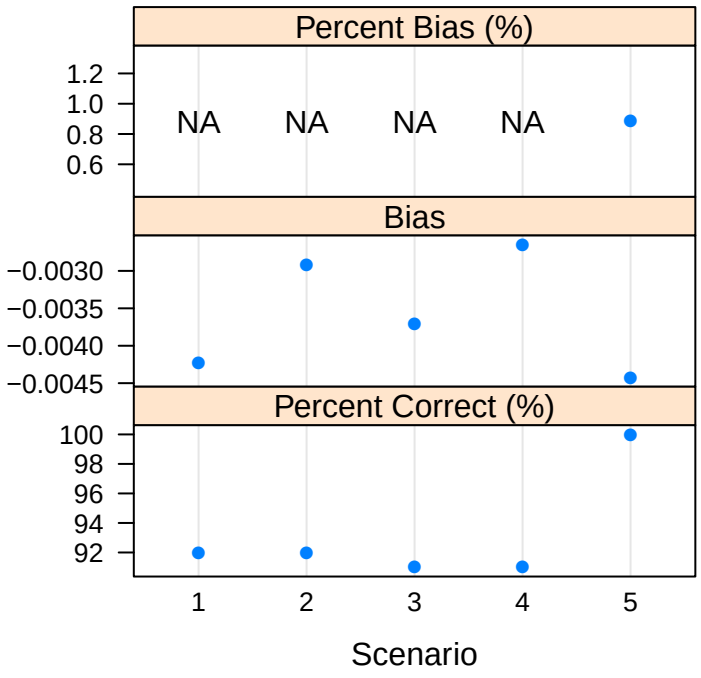

Figure 4.6: Summary of bias and classification for γ_{12}.

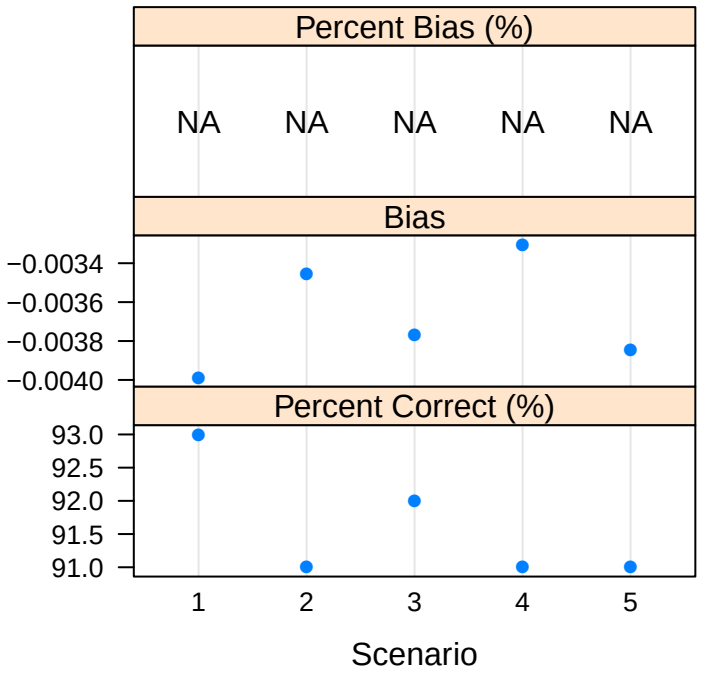

Figure 4.7: Summary of bias and classification for γ_{13}.

Table 4.1 through Table 4.5 describe in greater detail the results for all scenarios and all parameters. As the tables show, all of the parameters have been estimated fairly close to their true values, with small biases and generally small percent biases. The large percent biases for β_3 and β_5 are caused by those parameters being so close to zero, that even the relatively small biases become very large percent biases when the bias is divided by the true value.

Table 4.1: Results from first scenario, with all additive relations.

Parameter	True Value	Mean Estimate	Absolute Bias	Percent Bias	Mean Standard Error	Percent Antag.	Percent Additive	Percent Syn.
β_0	4.	4.012	0.01239	0.3	0.05435	NA	NA	NA
β_1	1.	0.9956	-0.004393	-0.4	0.01932	NA	NA	NA
β_2	0.6931	0.6782	-0.01497	-2.2	0.06543	NA	NA	NA
β_3	0.008	0.01666	0.008665	108.3	0.03008	NA	NA	NA
β_4	1.099	1.09	-0.008981	-0.8	0.05256	NA	NA	NA
β_5	0.005	0.01319	0.008187	163.7	0.03257	NA	NA	NA
γ_{12}	0.	-0.004222	-0.004222	NA	0.04816	7	92	1
γ_{13}	0.	-0.003987	-0.003987	NA	0.05064	5	93	2
γ_{23}	0.	0.0003786	0.0003786	NA	0.05652	1	99	0
γ_{123}	0.	0.01141	0.01141	NA	0.07471	2	91	7

Table 4.2: Results from second scenario, with 2-way synergy between Drug B and Drug C, but additivity for all other relations.

Parameter	True Value	Mean Estimate	Absolute Bias	Percent Bias	Mean Standard Error	Percent Antag.	Percent Additive	Percent Syn.
β_0	4.	4.011	0.01144	0.3	0.05872	NA	NA	NA
β_1	1.	0.9957	-0.004347	-0.4	0.02174	NA	NA	NA
β_2	0.6931	0.6808	-0.01235	-1.8	0.06596	NA	NA	NA
β_3	0.008	0.01568	0.007682	96.0	0.03152	NA	NA	NA
β_4	1.099	1.091	-0.007439	-0.7	0.05263	NA	NA	NA
β_5	0.005	0.01262	0.007622	152.4	0.0328	NA	NA	NA
γ_{12}	0.	-0.002921	-0.002921	NA	0.04991	7	92	1
γ_{13}	0.	-0.003455	-0.003455	NA	0.0516	6	91	3
γ_{23}	2.	2.02	0.01956	1.0	0.1605	0	0	100
γ_{123}	0.	0.006675	0.006675	NA	0.0949	0	94	6

Table 4.3: Results from third scenario, with 3-way synergy between Drug A, Drug B and Drug C, but additivity between each pair of drugs.

Parameter	True Value	Mean Estimate	Absolute Bias	Percent Bias	Mean Standard Error	Percent Antag.	Percent Additive	Percent Syn.
β_0	4.	4.014	0.0142	0.4	0.05687	NA	NA	NA
β_1	1.	0.9946	-0.005373	-0.5	0.02099	NA	NA	NA
β_2	0.6931	0.6768	-0.01634	-2.4	0.06764	NA	NA	NA
β_3	0.008	0.01805	0.01005	125.6	0.03269	NA	NA	NA
β_4	1.099	1.088	-0.0106	-1.0	0.05362	NA	NA	NA
β_5	0.005	0.015	0.01	200.0	0.03501	NA	NA	NA
γ_{12}	0.	-0.003711	-0.003711	NA	0.04877	8	91	1
γ_{13}	0.	-0.003768	-0.003768	NA	0.05106	5	92	3
γ_{23}	0.	0.00222	0.00222	NA	0.05838	1	99	0
γ_{123}	1.5	1.524	0.0242	1.6	0.0723	0	0	100

Table 4.4: Results from fourth scenario, with 2-way synergy between Drug B and Drug C, combined with 3-way synergy between Drug A, Drug B and Drug C.

Parameter	True Value	Mean Estimate	Absolute Bias	Percent Bias	Mean Standard Error	Percent Antag.	Percent Additive	Percent Syn.
β_0	4.	4.012	0.01248	0.3	0.05946	NA	NA	NA
β_1	1.	0.9951	-0.004905	-0.5	0.02249	NA	NA	NA
β_2	0.6931	0.6797	-0.01348	-1.9	0.06721	NA	NA	NA
β_3	0.008	0.01662	0.008621	107.8	0.0332	NA	NA	NA
β_4	1.099	1.09	-0.008415	-0.8	0.05302	NA	NA	NA
β_5	0.005	0.01367	0.008674	173.5	0.03433	NA	NA	NA
γ_{12}	0.	-0.002648	-0.002648	NA	0.05015	8	91	1
γ_{13}	0.	-0.003304	-0.003304	NA	0.0518	6	91	3
γ_{23}	2.	2.025	0.02465	1.2	0.1669	0	0	100
γ_{123}	1.5	1.521	0.0211	1.4	0.08548	0	0	100

Table 4.5: Results from fifth scenario, with 2-way antagonism between Drug A and Drug B, 2-way synergy between Drug B and Drug C, and 3-way synergy between the three drugs.

Parameter	True Value	Mean Estimate	Absolute Bias	Percent Bias	Mean Standard Error	Percent Antag.	Percent Additive	Percent Syn.
β_0	4.	4.012	0.01195	0.3	0.05899	NA	NA	NA
β_1	1.	0.9954	-0.004554	-0.5	0.0221	NA	NA	NA
β_2	0.6931	0.6801	-0.01303	-1.9	0.06576	NA	NA	NA
β_3	0.008	0.01593	0.007927	99.1	0.03181	NA	NA	NA
β_4	1.099	1.091	-0.007998	-0.7	0.05263	NA	NA	NA
β_5	0.005	0.01313	0.008131	162.6	0.03363	NA	NA	NA
γ_{12}	-0.5	-0.5044	-0.004423	0.9	0.03729	100	0	0
γ_{13}	0.	-0.003844	-0.003844	NA	0.0509	6	91	3
γ_{23}	2.	2.023	0.02291	1.1	0.1652	0	0	100
γ_{123}	1.5	1.516	0.01568	1.0	0.07742	0	0	100

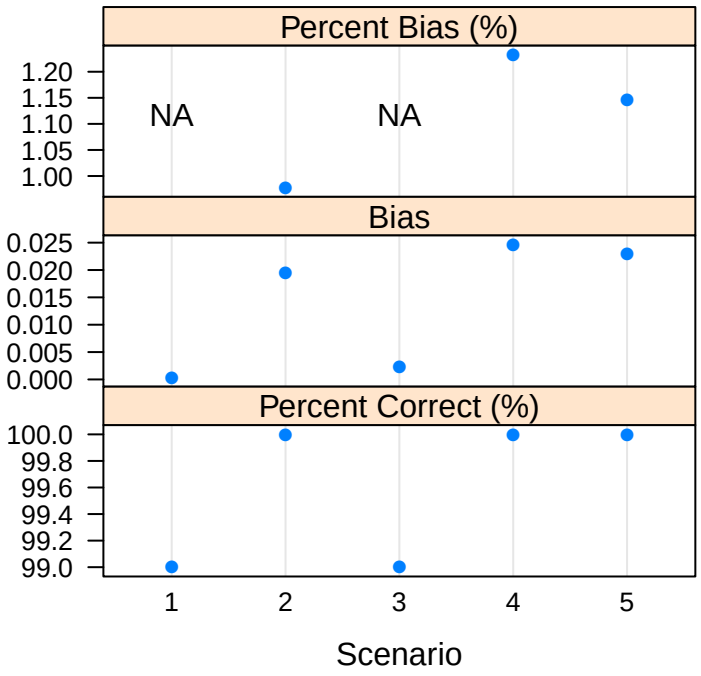

Figure 4.8: Summary of bias and classification for γ_{23}.

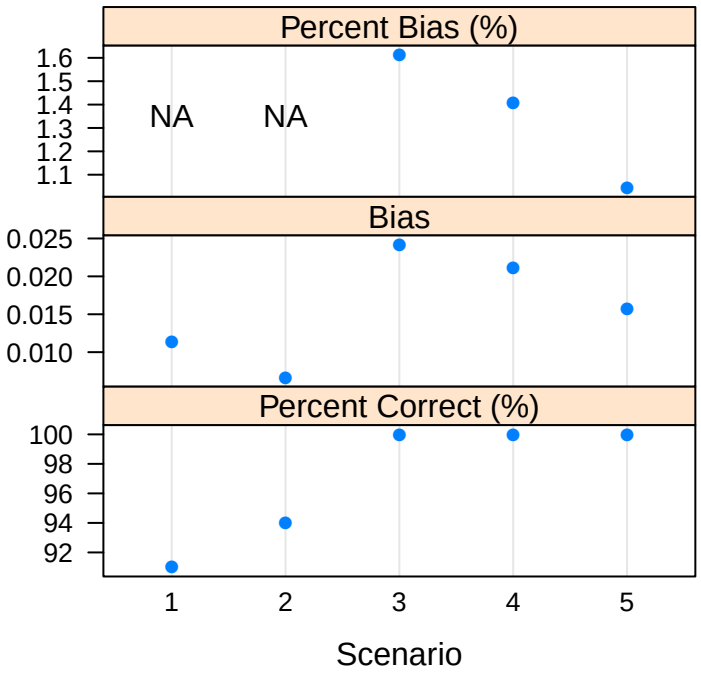

Figure 4.9: Summary of bias and classification for γ_{123}.

Chapter 5

Extend Kong and Lee's Parametric Model to Three or More Drugs

The next synergy method that this thesis extends to handle more than 2 drugs is Kong and Lee's parametric method, which was described in Section 2.4. As Kong and Lee's parametric method is a generalization of the Plummer and Short method, this extension is a generalization of the Plummer and Short extension proposed in Chapter 4.

This chapter will first describe a model for an extension of Kong and Lee's parametric method to n drugs. It will then describe in more detail the model for 3 drugs, and an implementation of the 3-drug model. The implementation of the 3-drug model will then be evaluated using simulated data.

5.1 General Model Description

The extended Kong and Lee parametric model begins with the same assumptions of the extended Plummer and Short method regarding the individual drugs, their individual responses, and their relative potencies to each other. See Section 4.1 for details.

An extended Kong and Lee parametric model for n drugs can then be defined as:

$$\begin{aligned} Y =& \beta_0 + \beta_1 \log \left(\sum_{j=1}^{n} \rho_j d_j \right. \\ & \left. + \sum_{p=2}^{n} \sum_{1 \le i_1 < i_2 < \cdots < i_p \le n} f_{i_1 i_2 \ldots i_p} \left(d_{i_1}, \ldots, d_{i_p}; \rho_{i_1}, \ldots, \rho_{i_p}, \kappa_{i_1 i_2 \ldots i_p} \right) \left(\prod_{j=1}^{p} \rho_{i_j} d_{i_j} \right)^{\frac{1}{p}} \right) \end{aligned} \tag{5.1}$$

where the relative potency parameters ρ_j are defined as previously and d_j is the amount of Drug j in the combination. In addition, i_1, i_2, ..., i_p are the drugs involved in a

p-way interaction, with respective doses of $d_{i_1}, \ldots, d_{i_p}$, and respective relative potencies to Drug 1 of $\rho_{i_1}, \ldots, \rho_{i_p}$.

The key difference between the extended Kong and Lee parametric model and the extended Plummer and Short model is that the fixed γ coefficients in the extended Plummer and Short model, which model the interactions between combinations of drugs, have been replaced by functions that allow the interactions to be modeled more flexibly. The f functions model the interactions between combinations of drugs, with the function $f_{i_1 i_2 \ldots i_p}$ modeling the p-way interaction between drugs $i_1, i_2, \ldots, i_p$. The κ vectors are vectors of parameters used in the f functions, with the vector $\kappa_{i_1 i_2 \ldots i_p}$ used in the function $f_{i_1 i_2 \ldots i_p}$. For all of the f functions in the model, positive values indicate synergy, negative values indicate antagonism, and a value of zero indicates additivity.

The f model functions and their κ parameter vectors allow more flexibility in modeling the interactions than is possible with the γ interaction coefficients in the extended Plummer and Short model. In the extended Plummer and Short model, a particular interaction is modeled by a scalar $\gamma_{i_1 i_2 \ldots i_p}$ so it must be consistent across all dose combinations; if the interaction is synergistic, the model assumes the interaction is synergistic at all dose combinations, and that it has the same strength of synergy at all dose combinations. In the extended Kong and Lee parametric model, the f model functions and their associated κ parameter vectors allow a particular interaction to vary in direction or strength at different dose combinations.

Each f function is a function of the doses involved in the interaction, their relative potencies, and the function's κ parameter vector. For the function $f_{i_1 i_2 \ldots i_p}$, modeling the p-way interaction between drugs $i_1, i_2, \ldots, i_p$, with relative potencies compared to

Drug 1 of $\rho_{i_1}, \ldots, \rho_{i_p}$, the following definition is proposed:

$$\begin{aligned} f_{i_1 i_2 \ldots i_p}\left(d_{i_1}, \ldots d_{i_p}; \rho_{i_1}, \ldots \rho_{i_p}, \kappa_{i_1 i_2 \ldots i_p}\right) = & \kappa_{i_1 i_2 \ldots i_p, 0} \\ & + \sum_{j=1}^{p} \kappa_{i_1 i_2 \ldots i_p, j} \left(\rho_{i_j} d_{i_j}\right)^{1/p} \\ & + \sum_{j=1}^{p} \kappa_{i_1 i_2 \ldots i_p, p+j} \left(\rho_{i_j} d_{i_j}\right) \\ & + \kappa_{i_1 i_2 \ldots i_p, 2p+1} \left(\prod_{j=1}^{p} \rho_{i_j} d_{i_j}\right)^{1/p} \end{aligned} \tag{5.2}$$

where the parameter vector $\kappa_{i_1 i_2 \ldots i_p} = \left(\kappa_{i_1 i_2 \ldots i_p, 0}, \ldots, \kappa_{i_1 i_2 \ldots i_p, 2p+i}\right)$.

The proposed functions only include polynomials of the individual drug doses and the geometric mean of all p drug doses, they do not include polynomial combinations of less than p drugs. The other polynomial combinations of less than p drugs are not included for a number of reasons. We believe the proposed functions already allow sufficient flexibility in modeling drug interactions. Any polynomial combinations of less than p drugs are already included in the f functions that model the j-way interactions, where $j < p$. Also, including the polynomial combinations of i drugs in f functions of j-way interactions, where $i < j$, may make it harder to estimate the polynomials across the multiple f functions, and increase the sample size required to adequately estimate them; there was some evidence of this in early experiments with a three-drug model that did include polynomial combinations of 2 drugs in the 3-way interaction function.

In the proposed functions, if the only non-zero parameters in all of the parameter vectors $\kappa_{i_1 i_2 \ldots i_p}$, $1 \leq i_1 < i_2 < \cdots < i_p \leq n$, are $\kappa_{i_1 i_2 \ldots i_p, 0}$, then the extended Kong and Lee parametric model reduces to an extended Plummer and Short model, with $\gamma_{i_1 i_2 \ldots i_p} = \kappa_{i_1 i_2 \ldots i_p, 0}$, for all $1 \leq i_1 < i_2 < \cdots < i_p \leq n$.

5.2 Three Drug Model Description

We assume there are three drugs, 1, 2, and 3. The extended Kong and Lee parametric model can be specified as:

$$\begin{aligned} Y = \beta_0 + \beta_1 \log(& d_1 + \rho_2 d_2 + \rho_3 d_3 \\ & + f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_{12}\right)\left(d_1\rho_2 d_2\right)^{1/2} + f_{13}\left(d_1, d_3; \beta_4, \beta 5, \kappa_{13}\right)\left(d_1\rho_3 d_3\right)^{1/2} \\ & + f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right)\left(\rho_2 d_2 \rho_3 d_3\right)^{1/2} \\ & + f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right)\left(d_1\rho_2 d_2\rho_3 d_3\right)^{1/3}) \end{aligned} \tag{5.3}$$

where κ_{12}, κ_{13}, κ_{23} and κ_{123} are vectors of parameters for modeling any local synergy and antagonism, and ρ_2 and ρ_3 are defined as in (4.2), and D_2 and D_3 are respectively the solutions to:

$$\begin{aligned} D_2 &= d_2 + d_1 e^{-\beta_2 - \beta_3 \log(D_2)} \quad (= d_2 + d_1/\rho_2) \\ D_3 &= d_3 + d_1 e^{-\beta_4 - \beta_5 \log(D_3)} \quad (= d_3 + d_1/\rho_3) \end{aligned}$$

Similar to the $f\left(d_1, d_2; \gamma\right)$ function in the original Kong and Lee parametric method, the functions $f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_{12}\right)$, $f_{13}\left(d_1, d_3; \beta_4, \beta_5, \kappa_{13}\right)$, $f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right)$, and $f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right)$ will be able to capture any local synergy or antagonism, not just global synergy or antagonism that the extended Plummer and Short method captures. The functions will be similar to the function used by the original Kong and Lee parametric method:

$$\begin{aligned} f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_{12}\right) =& \kappa_{12,0} + \kappa_{12,1} d_1^{\frac{1}{2}} + \kappa_{12,2}\left(\rho_2 d_2\right)^{\frac{1}{2}} + \kappa_{12,3} d_1 \\ & + \kappa_{12,4}\rho_2 d_2 + \kappa_{12,5}\left(d_1\rho_2 d_2\right)^{\frac{1}{2}} \end{aligned}$$

$$\begin{aligned} f_{13}\left(d_1, d_3; \beta_4, \beta_5, \kappa_{13}\right) =& \kappa_{13,0} + \kappa_{13,1} d_1^{\frac{1}{2}} + \kappa_{13,2}\left(\rho_3 d_3\right)^{\frac{1}{2}} + \kappa_{13,3} d_1 \\ & + \kappa_{13,4}\rho_3 d_3 + \kappa_{13,5}\left(d_1\rho_3 d_3\right)^{\frac{1}{2}} \end{aligned}$$

$$\begin{aligned} f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right) =& \kappa_{23,0} + \kappa_{23,1}\left(\rho_2 d_2\right)^{\frac{1}{2}} + \kappa_{23,2}\left(\rho_3 d_3\right)^{\frac{1}{2}} + \kappa_{23,3}\rho_2 d_2 \\ & + \kappa_{23,4}\rho_3 d_3 + \kappa_{23,5}\left(\rho_2 d_2 \rho_3 d_3\right)^{\frac{1}{2}} \end{aligned}$$

$$\begin{aligned} f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right) =& \kappa_{123,0} + \kappa_{123,1} d_1^{\frac{1}{3}} + \kappa_{123,2}\left(\rho_2 d_2\right)^{\frac{1}{3}} + \kappa_{123,3}\left(\rho_3 d_3\right)^{\frac{1}{3}} \\ &+ \kappa_{123,4} d_1 + \kappa_{123,5}\rho_2 d_2 + \kappa_{123,6}\rho_3 d_3 \\ &+ \kappa_{123,7}\left(d_1\rho_2 d_2\rho_3 d_3\right)^{\frac{1}{3}} \end{aligned}$$

As can be seen above, the extended Kong and Lee parametric method generalizes the extended Plummer and Short method by replacing the scalar parameters γ_{12}, γ_{13}, γ_{23} and γ_{123} in the extended Plummer and Short model in (4.3), with the more flexible functions f_{12}, f_{13}, f_{23} and f_{123} in the extended Kong and Lee parametric model in (5.3). If all κ parameters in the f_{12}, f_{13}, f_{23} and f_{123} functions are zero except for $\kappa_{12,0}$, $\kappa_{13,0}$, and $\kappa_{23,0}$ and $\kappa_{123,0}$, then the extended Plummer and Short model reduces to an extended Plummer and Short model, with $\gamma_{12} = \kappa_{12,0}$, $\gamma_{13} = \kappa_{13,0}$, $\gamma_{23} = \kappa_{23,0}$, and $\gamma_{123} = \kappa_{123,0}$.

5.2.1 Interaction Index Equivalence

It is possible to show the equivalence of the extended Kong and Lee parametric model, specified in (5.3), and the Interaction Index of the 3-drug Loewe additivity model, specified in (3.2). The derivation follows that used for the Extended Plummer and Short Model, in Section 4.2. using steps that follow those that Lee et al [21] used to show the equivalence of the Plummer and Short model and the 2-drug Loewe additivity model.

In (5.3), if $d_2 = d_3 = 0$, then $Y = \beta_0 + \beta_1 \log\left(d_1\right)$, and $\exp\left(\frac{Y-\beta_0}{\beta_1}\right) = D_{y,1}$ (recall from Section 3.3 that $D_{y,1}$ is the dose of drug 1 that would have to be used alone to have the same effect y). Similarly, if $d_1 = d_3 = 0$, then $Y = \beta_0 + \beta_1 \log\left(\rho_2 d_2\right)$, and $\rho_2^{-1}\exp\left(\frac{Y-\beta_0}{\beta_1}\right) = D_{y,2}$, while if $d_1 = d_2 = 0$, then $Y = \beta_0 + \beta_1 \log\left(\rho_3 d_3\right)$, and $\rho_3^{-1}\exp\left(\frac{Y-\beta_0}{\beta_1}\right) = D_{y,3}$.

Next, for notational simplicity, let

$$\begin{aligned} \Lambda =& f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_1\right)\left(d_1\rho_2 d_2\right)^{1/2} \\ &+ f_{13}\left(d_1, d_3; \beta_4, \beta_5, \kappa_2\right)\left(d_1\rho_3 d_3\right)^{1/2} \\ &+ f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_3\right)\left(\rho_2 d_2\rho_3 d_3\right)^{1/2} \\ &+ f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_0\right)\left(d_1\rho_2 d_2\rho_3 d_3\right)^{1/3} \end{aligned}$$

so (5.3) can be rewritten more compactly as

$$Y = \beta_0 + \beta_1 \log\left(d_1 + \rho_2 d_2 + \rho_3 d_3 + \Lambda\right)$$

The following steps can then be used to show the equivalence of (5.3) and the Interaction Index of the 3-drug Loewe Additivity Model (3.2).

$$\begin{aligned}
\exp\left(\frac{Y-\beta_0}{\beta_1}\right) &= d_1 + \rho_2 d_2 + \rho_3 d_3 + \Lambda \\
\exp\left(\frac{Y-\beta_0}{\beta_1}\right) - \Lambda &= d_1 + \rho_2 d_2 + \rho_3 d_3 \\
1 - \frac{\Lambda}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} &= \frac{d_1}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} + \frac{\rho_2 d_2}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} + \frac{\rho_3 d_3}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} \\
1 - \frac{\Lambda}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} &= \frac{d_1}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} + \frac{d_2}{\rho_2^{-1}\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} + \frac{d_3}{\rho_3^{-1}\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} \\
1 - \frac{\Lambda}{\exp\left(\frac{Y-\beta_0}{\beta_1}\right)} &= \frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}} + \frac{d_3}{D_{y,3}}
\end{aligned}$$

So, returning to the original notation, the Interaction Index of the Loewe additivity model is equivalent to

$$\begin{aligned}
II = 1 - \Big(& f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_1\right)\left(d_1 \rho_2 d_2\right)^{1/2} \\
& + f_{13}\left(d_1, d_3; \beta_4, \beta_5, \kappa_2\right)\left(d_1 \rho_3 d_3\right)^{1/2} \\
& + f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_3\right)\left(\rho_2 d_2 \rho_3 d_3\right)^{1/2} \\
& + f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_0\right)\left(d_1 \rho_2 d_2 \rho_3 d_3\right)^{1/3} \Big) \\
& \times \left(\exp\left(\frac{Y-\beta_0}{\beta_1}\right) \right)^{-1}
\end{aligned}$$

5.2.2 Model Fitting and Variance Estimation

The extended Kong and Lee parametric model in (5.3) is a nonlinear model that can be fitted using nonlinear regression. Because the β and κ parameters are estimated, their asymptotic properties follow the standard results from a nonlinear regression.

The synergy or antagonism at a specified combination dose (d_1, d_2, d_3) is determined by the sign and magnitude of the polynomial functions f_{12}, f_{13}, f_{23} and f_{123}. The variance of the functions f_{12}, f_{13}, f_{23}, and f_{123} can be estimated using the delta method.

Their estimated variance can then used to test the statistical significance of any synergy or antagonism estimated by f_{12}, f_{13}, f_{23} and f_{123}.

The rest of this sub-section shows how to estimate the variance of the f functions using the delta method, and how to calculate their confidence intervals. Dose combinations whose confidence interval does not include zero indicate significant synergy or antagonism.

For each combination dose (d_1, d_2), the variance of the estimated polynomial $f_{12}(d_1, d_2; \beta, \kappa)$ can be approximated using the delta method by $\widehat{\text{Var}}_{f_{12}} = \left(\frac{\partial f_{12}}{\partial(\beta,\kappa)}\right)' \Sigma \left(\frac{\partial f_{12}}{\partial(\beta,\kappa)}\right)|_{(\beta,\kappa)=\left(\hat{\beta},\hat{\kappa}\right)}$ where

$$\begin{aligned}\frac{\partial f_{12}}{\partial(\beta,\kappa)} &= \left(\frac{\partial f_{12}}{\partial\beta_2}, \frac{\partial f_{12}}{\partial\beta_3}, \frac{\partial f_{12}}{\partial\kappa_{12,0}}, \frac{\partial f_{12}}{\partial\kappa_{12,1}}, \frac{\partial f_{12}}{\partial\kappa_{12,2}}, \frac{\partial f_{12}}{\partial\kappa_{12,3}}, \frac{\partial f_{12}}{\partial\kappa_{12,4}}, \frac{\partial f_{12}}{\partial\kappa_{12,5}}\right)' \\ &= \left(\frac{\partial f_{12}}{\partial\rho_2}\frac{\partial\rho_2}{\partial\beta_2}, \frac{\partial f_{12}}{\partial\rho_3}\frac{\partial\rho_3}{\partial\beta_3}, 1, d_1^{\frac{1}{2}}, (\rho_2 d_2)^{\frac{1}{2}}, d_1, \rho_2 d_2, (d_1\rho_2 d_2)^{\frac{1}{2}}\right)'\end{aligned}$$

with $\frac{\partial f_{12}}{\partial\rho_2} = \frac{1}{2}\kappa_{12,2}\left(\frac{d_2}{\rho_2}\right)^{\frac{1}{2}} + \kappa_{12,4}d_2 + \frac{1}{2}\kappa_{12,5}\left(\frac{d_1 d_2}{\rho_2}\right)^{\frac{1}{2}}$, $\frac{\partial\rho_2}{\partial\beta_2} = \frac{\rho_2^2\left(d_2+d_1\rho_2^{-1}\right)}{\rho_2\left(d_2+d_1\rho_2^{-1}\right)+\beta_3 d_1}$, and $\frac{\partial\rho_2}{\partial\beta_3} = \frac{\rho_2^2\left(d_2+d_1\rho_2^{-1}\right)}{\rho_2\left(d_2+d_1\rho_2^{-1}\right)+\beta_3 d_1}\log\left(d_2+d_1\rho_2^{-1}\right)$. Σ is the estimated covariance matrix of the parameters $(\beta_2, \beta_3, \kappa_{12,0}, \kappa_{12,1}, \kappa_{12,2}, \kappa_{12,3}, \kappa_{12,4}, \kappa_{12,5})$. So the $(1-\alpha)\times 100\%$ upper and lower confidence surfaces for $f_{12}(d_1, d_2; \beta, \kappa)$ can be constructed as:

$$f_{12_{l,u}}(d_1, d_2) = \widehat{f_{12}}(d_1, d_2) \pm t_{\frac{\alpha}{2},n-p}\sqrt{\widehat{\text{Var}}_{f_{12}}(d_1, d_2)},$$

where $t_{\frac{\alpha}{2},n-p}$ is the upper $\frac{\alpha}{2}$ percentile of a t-distribution with $n-p$ degrees of freedom.

For each combination dose (d_1, d_3), the variance of the estimated polynomial $f_{13}(d_1, d_3; \beta, \kappa)$ can be approximated using the delta method by $\widehat{\text{Var}}_{f_{13}} = \left(\frac{\partial f_{13}}{\partial(\beta,\kappa)}\right)' \Sigma \left(\frac{\partial f_{13}}{\partial(\beta,\kappa)}\right)|_{(\beta,\kappa)=\left(\hat{\beta},\hat{\kappa}\right)}$ where

$$\begin{aligned}\frac{\partial f_{13}}{\partial(\beta,\kappa)} &= \left(\frac{\partial f_{13}}{\partial\beta_4}, \frac{\partial f_{13}}{\partial\beta_5}, \frac{\partial f_{13}}{\partial\kappa_{13,0}}, \frac{\partial f_{13}}{\partial\kappa_{13,1}}, \frac{\partial f_{13}}{\partial\kappa_{13,2}}, \frac{\partial f_{13}}{\partial\kappa_{13,3}}, \frac{\partial f_{13}}{\partial\kappa_{13,4}}, \frac{\partial f_{13}}{\partial\kappa_{13,5}}\right)' \\ &= \left(\frac{\partial f_{13}}{\partial\rho_3}\frac{\partial\rho_3}{\partial\beta_4}, \frac{\partial f_{13}}{\partial\rho_3}\frac{\partial\rho_3}{\partial\beta_5}, 1, d_1^{\frac{1}{2}}, (\rho_3 d_3)^{\frac{1}{2}}, d_1, \rho_3 d_3, (d_1\rho_3 d_3)^{\frac{1}{2}}\right)'\end{aligned}$$

with $\frac{\partial f_{13}}{\partial\rho_3} = \frac{1}{2}\kappa_{13,2}\left(\frac{d_3}{\rho_3}\right)^{\frac{1}{2}} + \kappa_{13,4}d_3 + \frac{1}{2}\kappa_{13,5}\left(\frac{d_1 d_3}{\rho_3}\right)^{\frac{1}{2}}$, $\frac{\partial\rho_3}{\partial\beta_4} = \frac{\rho_3^2\left(d_3+d_1\rho_3^{-1}\right)}{\rho_3\left(d_3+d_1\rho_3^{-1}\right)+\beta_5 d_1}$, and $\frac{\partial\rho_3}{\partial\beta_5} = \frac{\rho_3^2\left(d_3+d_1\rho_3^{-1}\right)}{\rho_3\left(d_3+d_1\rho_3^{-1}\right)+\beta_5 d_1}\log\left(d_3+d_1\rho_3^{-1}\right)$. Σ is the estimated covariance matrix of the parameters $(\beta_4, \beta_5, \kappa_{13,0}, \kappa_{13,1}, \kappa_{13,2}, \kappa_{13,3}, \kappa_{13,4}, \kappa_{13,5})$. So the $(1-\alpha)\times 100\%$ upper and

lower confidence surfaces for $f_{13}(d_1, d_3; \beta, \kappa)$ can be constructed as:

$$f_{13_{l,u}}(d_1, d_3) = \widehat{f_{13}}(d_1, d_3) \pm t_{\frac{\alpha}{2}, n-p} \sqrt{\widehat{\text{Var}}_{f_{13}}(d_1, d_3)},$$

where $t_{\frac{\alpha}{2}, n-p}$ is the upper $\frac{\alpha}{2}$ percentile of a t-distribution with $n-p$ degrees of freedom.

For each combination dose (d_2, d_3), the variance of the estimated polynomial $f_{23}(d_2, d_3; \beta, \kappa)$ can be approximated using the delta method by $\widehat{\text{Var}}_{f_{23}} = \left(\frac{\partial f_{23}}{\partial(\beta,\kappa)}\right)' \Sigma \left(\frac{\partial f_{23}}{\partial(\beta,\kappa)}\right)|_{(\beta,\kappa)=(\hat{\beta},\hat{\kappa})}$ where

$$\begin{aligned}
\frac{\partial f_{23}}{\partial(\beta,\kappa)} &= \left(\frac{\partial f_{23}}{\partial\beta_2}, \frac{\partial f_{23}}{\partial\beta_3}, \frac{\partial f_{23}}{\partial\beta_4}, \frac{\partial f_{23}}{\partial\beta_5}, \frac{\partial f_{23}}{\partial\kappa_{23,0}}, \frac{\partial f_{23}}{\partial\kappa_{23,1}}, \frac{\partial f_{23}}{\partial\kappa_{23,2}}, \frac{\partial f_{23}}{\partial\kappa_{23,3}}, \frac{\partial f_{23}}{\partial\kappa_{23,4}}, \frac{\partial f_{23}}{\partial\kappa_{23,5}}\right)' \\
&= \left(\frac{\partial f_{23}}{\partial\rho_2}\frac{\partial\rho_2}{\partial\beta_2}, \frac{\partial f_{23}}{\partial\rho_2}\frac{\partial\rho_2}{\partial\beta_3}, \frac{\partial f_{23}}{\partial\rho_3}\frac{\partial\rho_3}{\partial\beta_4}, \frac{\partial f_{23}}{\partial\rho_3}\frac{\partial\rho_3}{\partial\beta_5}, 1, (\rho_2 d_2)^{\frac{1}{2}}, (\rho_3 d_3)^{\frac{1}{2}}, \rho_2 d_2, \rho_3 d_3,\right. \\
&\qquad \left.(\rho_2 d_2 \rho_3 d_3)^{\frac{1}{2}}\right)'
\end{aligned}$$

with $\frac{\partial f_{23}}{\partial\rho_2} = \frac{1}{2}\kappa_{23,1}\left(\frac{d_2}{\rho_2}\right)^{\frac{1}{2}} + \kappa_{23,3}d_2 + \frac{1}{2}\kappa_{23,5}\left(\frac{d_2\rho_3 d_3}{\rho_2}\right)^{\frac{1}{2}}$, $\frac{\partial f_{23}}{\partial\rho_3} = \frac{1}{2}\kappa_{23,2}\left(\frac{d_3}{\rho_3}\right)^{\frac{1}{2}} + \kappa_{23,4}d_3 + \frac{1}{2}\kappa_{23,5}\left(\frac{\rho_2 d_2 d_3}{\rho_3}\right)^{\frac{1}{2}}$, and $\frac{\partial\rho_2}{\partial\beta_2}$, $\frac{\partial\rho_2}{\partial\beta_3}$, $\frac{\partial\rho_3}{\partial\beta_4}$, and $\frac{\partial\rho_3}{\partial\beta_5}$ defined as shown above. Σ is the estimated covariance matrix of the parameters $(\beta_2,\ \beta_3, \beta_4, \beta_5, \kappa_{23,0}, \kappa_{23,1}, \kappa_{23,2}, \kappa_{23,3}, \kappa_{23,4},\ \kappa_{23,5})$. So the $(1-\alpha)\times 100\%$ upper and lower confidence surfaces for $f_{23}(d_2, d_3; \beta, \kappa)$ can be constructed as:

$$f_{23_{l,u}}(d_2, d_3) = \widehat{f_{23}}(d_2, d_3) \pm t_{\frac{\alpha}{2}, n-p} \sqrt{\widehat{\text{Var}}_{f_{23}}(d_2, d_3)},$$

where $t_{\frac{\alpha}{2}, n-p}$ is the upper $\frac{\alpha}{2}$ percentile of a t-distribution with $n-p$ degrees of freedom.

For each combination dose (d_1, d_2, d_3), the variance of the estimated polynomial $f_{123}(d_1, d_2, d_3; \beta, \kappa)$ can be approximated using the delta method by $\widehat{\text{Var}}_{f_{123}} = \left(\frac{\partial f_{123}}{\partial(\beta,\kappa)}\right)' \Sigma \left(\frac{\partial f_{123}}{\partial(\beta,\kappa)}\right)|_{(\beta,\kappa)=(\hat{\beta},\hat{\kappa})}$ where

$$\begin{aligned}
\frac{\partial f_{123}}{\partial(\beta,\kappa)} &= \left(\frac{\partial f_{123}}{\partial\beta_2}, \frac{\partial f_{123}}{\partial\beta_3}, \frac{\partial f_{123}}{\partial\beta_4}, \frac{\partial f_{123}}{\partial\beta_5}, \frac{\partial f_{123}}{\partial\kappa_{123,0}}, \frac{\partial f_{123}}{\partial\kappa_{123,1}},\right. \\
&\qquad \left.\frac{\partial f_{123}}{\partial\kappa_{123,2}}, \frac{\partial f_{123}}{\partial\kappa_{123,3}}, \frac{\partial f_{123}}{\partial\kappa_{123,4}}, \frac{\partial f_{123}}{\partial\kappa_{123,5}}, \frac{\partial f_{123}}{\partial\kappa_{123,6}}, \frac{\partial f_{123}}{\partial\kappa_{123,7}}\right)' \\
&= \left(\frac{\partial f_{123}}{\partial\rho_2}\frac{\partial\rho_2}{\partial\beta_2}, \frac{\partial f_{123}}{\partial\rho_2}\frac{\partial\rho_2}{\partial\beta_3}, \frac{\partial f_{123}}{\partial\rho_3}\frac{\partial\rho_3}{\partial\beta_4}, \frac{\partial f_{123}}{\partial\rho_3}\frac{\partial\rho_3}{\partial\beta_5}, 1, (d_1)^{\frac{1}{3}},\right. \\
&\qquad \left.(\rho_2 d_2)^{\frac{1}{3}}, (\rho_3 d_3)^{\frac{1}{3}}, d_1, \rho_2 d_2, \rho_3 d_3, (d_1 \rho_2 d_2 \rho_3 d_3)^{\frac{1}{3}}\right)'
\end{aligned}$$

with $\frac{\partial f_{123}}{\partial \rho_2} = \frac{1}{3}\kappa_{123,2}\left(\frac{d_2}{\rho_2^2}\right)^{\frac{1}{3}} + \kappa_{123,5}d_2 + \frac{1}{3}\kappa_{123,7}\left(\frac{d_1 d_2 \rho_3 d_3}{\rho_2^2}\right)^{\frac{1}{3}}$, $\frac{\partial f_{123}}{\partial \rho_3} = \frac{1}{3}\kappa_{123,3}\left(\frac{d_3}{\rho_3^2}\right)^{\frac{1}{3}} + \kappa_{123,6}d_3 + \frac{1}{3}\kappa_{123,7}\left(\frac{d_1 \rho_2 d_2 d_3}{\rho_3^2}\right)^{\frac{1}{3}}$, and $\frac{\partial \rho_2}{\partial \beta_2}$, $\frac{\partial \rho_2}{\partial \beta_3}$, $\frac{\partial \rho_3}{\partial \beta_4}$, and $\frac{\partial \rho_3}{\partial \beta_5}$ defined as shown above. Σ is the estimated covariance matrix of the parameters $(\beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123,0}, \kappa_{123,1}, \kappa_{123,2}, \kappa_{123,3}, \kappa_{123,4}, \kappa_{123,5}, \kappa_{123,6}, \kappa_{123,7})$. So the $(1-\alpha)\times 100\%$ upper and lower confidence surfaces for $f_{123}(d_1, d_2, d_3; \beta, \kappa)$ can be constructed as:

$$f_{123_{l,u}}(d_1, d_2, d_3) = \widehat{f_{123}}(d_1, d_2, d_3) \pm t_{\frac{\alpha}{2}, n-p}\sqrt{\widehat{\mathrm{Var}}_{f_{123}}(d_1, d_2, d_3)},$$

where $t_{\frac{\alpha}{2}, n-p}$ is the upper $\frac{\alpha}{2}$ percentile of a t-distribution with $n-p$ degrees of freedom.

5.2.3 Model Building Algorithm

Because of the large number of parameters, a model building algorithm, similar to that in Kong and Lee, will be used to attempt to reduce the model to its essential parameters.

After fitting the full model, the parameter with the smallest t value will be considered for removal, subject to the following restrictions. No "main effect" parameter will be removed from the model as long as any of its interaction terms remain in the model. Also, none of the β parameters will be considered for removal, nor will $\kappa_{12,0}$, $\kappa_{13,0}$, $\kappa_{23,0}$, or $\kappa_{123,0}$. After the candidate parameter for removal is chosen, a new model excluding that parameter will be fitted. The Akaike Information Criterion (AIC) for the reduced model will then be compared to the AIC from the original model. If the AIC of the reduced model is better (lower) than the AIC of the original model, then the candidate parameter is removed from the model, and the process repeats, considering another parameter for removal. If the AIC of the reduced model is worse (higher) than the AIC of the original model, then the model building algorithm concludes and the original model is selected as the "final" model. If all candidate parameters are removed, the model will have been reduced to the extended Plummer and Short model.

The variance estimations described in Section 5.2.2 were based on the full extended Kong and Lee parametric model. The variance estimation based on the final model is done similarly.

5.3 Response Surface Estimation

An R program was created to implement the extended Kong and Lee parametric method described in the Section 5.2.

Because no data sets were available for analysis, simulated data sets were used. Two data sets were constructed and analyzed using the program. The two constructed data sets corresponded to 2 different synergy scenarios.

In the first scenario there was 2-way synergy between one pair of drugs and also 3-way synergy between all of the drugs. This scenario evaluates how well the model can distinguish between the two types of synergy.

In the second scenario there was localized 3-way synergy and 3-way antagonism. This scenario evaluates how well the model handles synergistic relationships which could not be adequately modeled by the extended Plummer and Short model.

5.3.1 Simulation Data Construction

For both synergy scenarios, each constructed data set consisted of generated measurements for combination treatments of 3 drugs. All 3 drugs had 7 dose levels. In addition to a dose of 0, the first drug had dose levels of 2.5, 5, 7.5, 10, 15, and 20. The second drug had levels one half of the first drug, while the third had levels one quarter of the first drug. At each unique dose combination, 3 repetitions were simulated.

The relative potency of the first drug to the second drug was defined as ρ_2:

$$\log(\rho_2) = \beta_2 + \beta_3 \log(D_2) \tag{5.4}$$

where D_2 is the solution to:

$$D_2 = d_2 + d_1/\rho_2$$

and $\beta_2 = \log 2$ and $\beta_3 = 0.02$. The relative potency of the first drug to the third drug was defined as ρ_3:

$$\log(\rho_3) = \beta_4 + \beta_5 \log(D_3) \tag{5.5}$$

where D_3 is the solution to:

$$D_3 = d_3 + d_1/\rho_3$$

and $\beta_4 = \log 3$ and $\beta_5 = 0.005$. All possible combinations of the 3 drugs at the given dose levels were used, with three generated results at each combination.

The generated response variable was a proportion, such as the proportion of cells viable after the given treatment, a common end point in many nonclinical oncology studies. Because the analysis program assumes the response E is a proportion, it initially transforms it to be the transformed effect Y using the function $g(E) = \operatorname{logit} E = \log \frac{E}{1-E}$. All generated responses were constructed on the transformed scale as y, and then transformed to be E on the original scale of responses using the function $g^{-1}(y) = \operatorname{logit}^{-1} y = \frac{1}{1+\exp(-y)}$. A small amount of random noise, from a normal distribution with a mean of 0 and a standard deviation of 0.1, was added to each generated response value before its antilogit transformation.

The constructed data set for the first scenario assumed that there was 2-way synergy between one pair of drugs and 3-way synergy between all 3 drugs. The response was constructed as:

$$\begin{aligned} Y = \beta_0 + \beta_1 \log(& d_1 + \rho_2 d_2 + \rho_3 d_3 \\ & + f_{12}(d_1, d_2; \beta_2, \beta_3, \kappa_1)(d_1 \rho_2 d_2)^{1/2} \\ & + f_{123}(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_0)(d_1 \rho_2 d_2 \rho_3 d_3)^{1/3}) + \epsilon \end{aligned}$$

where:

$$\begin{aligned} f_{12}(d_1, d_2; \beta_2, \beta_3, \kappa_1) =& \kappa_{12,0} + \kappa_{12,1} d_1^{\frac{1}{2}} + \kappa_{12,2}(\rho_2 d_2)^{\frac{1}{2}} + \kappa_{12,3} d_1 \\ & + \kappa_{12,4} \rho_2 d_2 + \kappa_{12,5}(d_1 \rho_2 d_2)^{\frac{1}{2}} \end{aligned}$$

$$\begin{aligned} f_{123}(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_0) =& \kappa_{123,0} + \kappa_{123,1} d_1^{\frac{1}{3}} + \kappa_{123,2}(\rho_2 d_2)^{\frac{1}{3}} + \kappa_{123,3}(\rho_3 d_3)^{\frac{1}{3}} \\ & + \kappa_{123,4} d_1 + \kappa_{123,5} \rho_2 d_2 + \kappa_{123,6} \rho_3 d_3 \\ & + \kappa_{123,7}(d_1 \rho_2 d_2 \rho_3 d_3)^{\frac{1}{3}} \\ & \epsilon \sim N\left(0, 0.1^2\right) \end{aligned}$$

where $\beta_0 = 4$, $\beta_1 = -1$, ρ_2 and ρ_3 were defined as in (5.4) and (5.5) respectively, with $\beta_2 = \log 2$, $\beta_3 = 0.02$, $\beta_4 = \log 3$, $\beta_5 = 0.005$, $\kappa_{12,0} = 0.4$, $\kappa_{123,7} = 0.08$, and all other parameters were 0.

The constructed data set for the second scenario assumed that there was local 3-way synergy between all 3 drugs in one region, but local 3-way antagonism between all 3 drugs in another region; all 2-way relations were assumed to be additive. The response was constructed as: The response was constructed as:

$$Y = \beta_0 + \beta_1 \log(d_1 + \rho_2 d_2 + \rho_3 d_3 + f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_0\right)\left(d_1 \rho_2 d_2 \rho_3 d_3\right)^{1/3}) + \epsilon$$

where:

$$\begin{aligned} f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_0\right) =& \kappa_{123,0} + \kappa_{123,1} d_1^{\frac{1}{3}} + \kappa_{123,2}\left(\rho_2 d_2\right)^{\frac{1}{3}} + \kappa_{123,3}\left(\rho_3 d_3\right)^{\frac{1}{3}} \\ &+ \kappa_{123,4} d_1 + \kappa_{123,5} \rho_2 d_2 + \kappa_{123,6} \rho_3 d_3 \\ &+ \kappa_{123,7}\left(d_1 \rho_2 d_2 \rho_3 d_3\right)^{\frac{1}{3}} \\ \epsilon \sim & N\left(0, 0.1^2\right) \end{aligned}$$

where $\beta_0 = 4$, $\beta_1 = -1$, ρ_2 and ρ_3 were defined as in (5.4) and (5.5) respectively, with $\beta_2 = \log 2$, $\beta_3 = 0.02$, $\beta_4 = \log 3$, $\beta_5 = 0.005$, $\kappa_{123,0} = 1$, $\kappa_{123,3} = 0.5$, $\kappa_{123,7} = -0.2$, and all other parameters were 0.

5.3.2 First Scenario: Two-Way Synergy and Three-Way Synergy

The full fitted model for the first scenario data is shown in Table 5.1. It shows that $\kappa_{123,7}$ was the only significant κ parameter in the model, and it was estimated slightly over 50% above its true value. $\kappa_{12,0}$, the only other κ parameter with a non-zero true value, was not significant, and it was also estimated about 50% above its true value.

Although $\kappa_{12,0}$ was not significant, nor were any of the other κ parameters in the f_{12} function, the confidence interval for the f_{12} function itself was significantly different from zero at most dose combinations, as shown in Panel R of Figure 5.1. This indicates that the model did detect the synergy between Drugs 1 and Drugs 2, even though the individual parameter estimates were not significant.

For the final model, the model building algorithm removed all of the κ parameters considered for removal, except for $\kappa_{123,7}$. The remaining κ parameters were retained in the model because of the model building rules, although some of them would have been

Table 5.1: Estimated parameters of full model for Scenario 1

Parameter	True Value	Estimated Value	Standard Error	p-value
β_0	4.	3.988	0.0715	<0.001
β_1	-1.	-0.993	0.0321	<0.001
β_2	0.693	0.677	0.0724	<0.001
β_3	0.02	0.036	0.046	0.430
β_4	1.099	1.114	0.0513	<0.001
β_5	0.005	-0.015	0.045	0.734
$\kappa_{12,0}$	0.4	0.611	0.3759	0.104
$\kappa_{12,1}$	0.	-0.16	0.1794	0.373
$\kappa_{12,2}$	0.	0.043	0.1683	0.799
$\kappa_{12,3}$	0.	0.026	0.0283	0.354
$\kappa_{12,4}$	0.	-0.01	0.0251	0.680
$\kappa_{12,5}$	0.	-0.001	0.0201	0.976
$\kappa_{13,0}$	0.	-0.468	0.3472	0.178
$\kappa_{13,1}$	0.	0.251	0.1587	0.115
$\kappa_{13,2}$	0.	0.1	0.1925	0.602
$\kappa_{13,3}$	0.	-0.029	0.0247	0.238
$\kappa_{13,4}$	0.	-0.021	0.0342	0.543
$\kappa_{13,5}$	0.	-0.009	0.0245	0.708
$\kappa_{23,0}$	0.	-0.033	0.3386	0.922
$\kappa_{23,1}$	0.	-0.059	0.1451	0.684
$\kappa_{23,2}$	0.	0.059	0.1872	0.753
$\kappa_{23,3}$	0.	0.013	0.0223	0.572
$\kappa_{23,4}$	0.	0.009	0.0342	0.791
$\kappa_{23,5}$	0.	-0.02	0.024	0.408
$\kappa_{123,0}$	0.	1.606	1.0768	0.136
$\kappa_{123,1}$	0.	-0.465	0.4474	0.299
$\kappa_{123,2}$	0.	-0.329	0.4238	0.438
$\kappa_{123,3}$	0.	-0.351	0.4621	0.447
$\kappa_{123,4}$	0.	0.011	0.034	0.746
$\kappa_{123,5}$	0.	0.015	0.0305	0.632
$\kappa_{123,6}$	0.	0.013	0.0411	0.749
$\kappa_{123,7}$	0.08	0.125	0.0441	0.005

retained anyway due to their significance. The final fitted model for the first scenario data is shown in Table 5.1. As shown in the table, $\kappa_{123,7}$ is still significant, although it is estimated almost 40% above its true value. $\kappa_{12,0}$, the only other κ parameter with a non-zero true value, is now significant, and is estimated only less than 10% above its true value. This appears to be one case where the final model performs better than the full model, although the goodness of fit evaluations showed in general that the full model usually performs better than the final model.

Table 5.2: Estimated parameters of final model for Scenario 1

Parameter	True Value	Estimated Value	Standard Error	p-value
β_0	4.	3.988	0.054	<0.001
β_1	-1.	-0.994	0.0221	<0.001
β_2	0.693	0.692	0.0596	<0.001
β_3	0.02	0.02	0.0311	0.514
β_4	1.099	1.103	0.0434	<0.001
β_5	0.005	0.013	0.0323	0.690
$\kappa_{12,0}$	0.4	0.429	0.0567	<0.001
$\kappa_{13,0}$	0.	-0.014	0.1029	0.889
$\kappa_{13,1}$	0.	0.05	0.0293	0.086
$\kappa_{13,2}$	0.	-0.055	0.03	0.069
$\kappa_{23,0}$	0.	-0.027	0.0501	0.594
$\kappa_{123,0}$	0.	0.88	0.486	0.070
$\kappa_{123,1}$	0.	-0.286	0.1253	0.023
$\kappa_{123,2}$	0.	-0.176	0.1188	0.138
$\kappa_{123,3}$	0.	-0.077	0.1337	0.564
$\kappa_{123,7}$	0.08	0.11	0.0264	<0.001

Figure 5.1 graphically shows the results for the first constructed data set. Panels A through C of the figure show the fitted log dose-response curves for each individual drug, used alone. The log-dose response curves were used to construct initial estimates for the extended Kong and Lee parametric model.

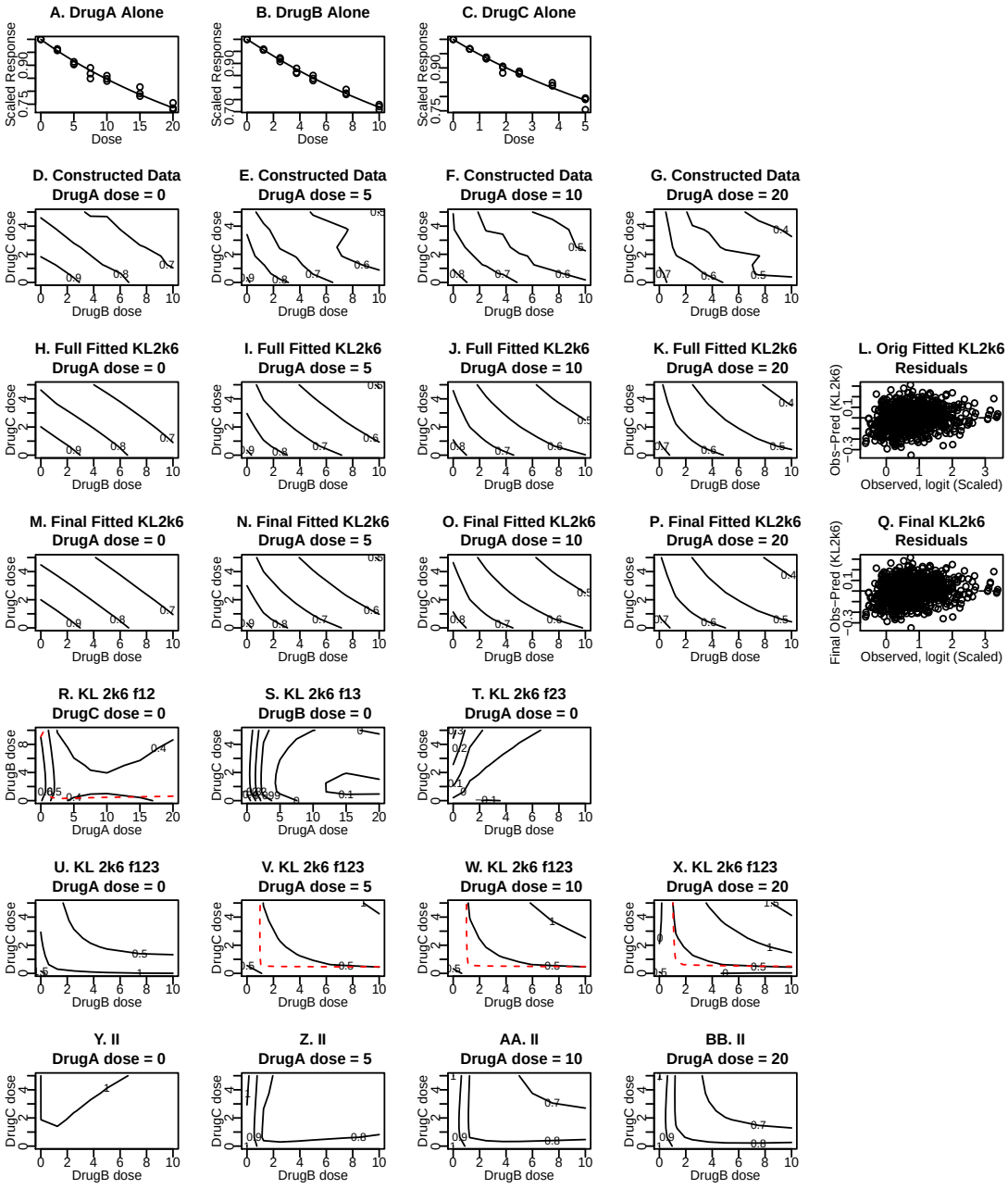

Figure 5.1: Results from first constructed data set, with 2-way synergy between Drug A and Drug B, and 3-way synergy between Drug A, Drug B and Drug C.

Most of the remaining plots show contour plots of various models and functions, but many of these are 3-dimensional figures in 4-dimensional space. So they are shown using multiple contour plots of 3-dimensional figures, for different levels along the fourth dimension. The dose level of Drug A is treated as the fourth dimension, so there are separate figures for different dose levels of Drug A. Within each plot, the contours show the response for the given doses of Drug B and Drug C on the X and Y axes respectively.

Panels D through G in the figure show contour plots of the "observed" constructed data. The contour plots of the "observed" data in Panels E through G are generally curved downward, reflecting the the 3-way synergy between Drug A, Drug B and Drug C. Some of the lines are rather jagged, due to the variability in the data. The lines in Panel D are more straight, reflecting the fact that this is 3-way synergy that is not present when Drug A is not present. The 2-way synergy between Drug A and Drug B is difficult to discern in the plots because it does not occur along the X and Y axes of the individual plots, but rather across the X axes of all of the plots.

Panels H through K show contour plots of the full fitted model. Panels I through K are curved downward, reflecting the 3-way synergy, and have smoothed out the jaggedness in the observed data plots. Panel L shows the residuals of the full fitted model, plotted against the observed (logit) response.

Panels M through P show contour plots of the final fitted model. There appears to be little difference between the plots of the final model and the full model. Panel Q shows the residuals of the final fitted model, which do not appear to be very different from those of the full fitted model, shown in Panel L.

Panel R shows a contour plot of the f_{12} function of the full fitted model. The dashed red line shows where the upper 95% confidence interval for the mean crosses the zero plane. This indicates the border of the region of statistical significant synergy between Drug A and Drug B, which is present and significant at most dose combinations.

Panel S shows a contour plot of the f_{13} function of the full fitted model. Ideally the plot would be perfectly flat on the zero plane, reflecting the true value zero of f_{13}. Although the positive contour lines surface indicate that synergy is present, the lack of a dashed red line indicates that it is not statistically significant at any dose combination.

Panel T shows a contour plot of the f_{23} function of the full fitted model. As with the plot of f_{13}, ideally this plot would be perfectly flat on the zero plane, reflecting the true value zero of f_{23}. And as with f_{13}, although the positive contour lines surface indicate that synergy is present, the lack of a dashed red line indicates that it is not statistically significant at any dose combination.

Panels U through X show contour plots of the f_{123} function of the full fitted model. The positive contour lines indicate that synergy is present, and the dashed red lines indicate that it is statistically significant at most dose combinations, except where Drug A is absent.

Panels Y through BB show contour plots of the Interaction Index based on the full fitted model. The contour lines less than 1 indicate that synergy is present at most dose combinations, except where Drug A is absent.

5.3.3 Second Scenario: Local Three-Way Synergy and Antagonism

The full fitted model for the second scenario data is shown in Table 5.3. As shown in the table, $\kappa_{123,7}$ was the only significant κ parameters in the model. $\kappa_{123,0}$ and $\kappa_{123,3}$ were over-estimated by approximately 20%, although $\kappa_{123,7}$ was estimated within 5% of its true value.

Although $\kappa_{123,7}$ was the only significant parameter in the f_{123} function, the model did find significant 3-way synergy and antagonism, as shown in Panels V through X of Figure 5.2. This indicates that the model did detect the 3-way local synergy and local antagonism between the drugs, even though only one of the individual parameter estimates was significant.

The final fitted model for the second scenario data is shown in Table 5.4. Of the κ parameters considered for removal that had a true value of zero, only some were removed by the model building algorithm. $\kappa_{13,1}$ and $\kappa_{23,2}$ were left in the model, and both of them were significant. significance. Of the three κ parameters with true non-zero values, $\kappa_{123,0}$ is not significant, but both $\kappa_{123,3}$ and $\kappa_{123,7}$ are significant. $\kappa_{123,0}$ is now underestimated by over 50%, while $\kappa_{123,3}$ is overestimated by 50%, and $\kappa_{123,7}$ is underestimated by about 20%.

Table 5.3: Estimated parameters of full model for Scenario 2

Parameter	True Value	Estimated Value	Standard Error	p-value
β_0	4.	3.978	0.0759	<0.001
β_1	-1.	-0.987	0.0339	<0.001
β_2	0.693	0.677	0.0772	<0.001
β_3	0.02	0.022	0.0485	0.645
β_4	1.099	1.112	0.0551	<0.001
β_5	0.005	-0.006	0.0483	0.907
$\kappa_{12,0}$	0.	-0.106	0.3414	0.755
$\kappa_{12,1}$	0.	-0.033	0.1642	0.843
$\kappa_{12,2}$	0.	0.09	0.1588	0.571
$\kappa_{12,3}$	0.	0.015	0.0262	0.566
$\kappa_{12,4}$	0.	-0.007	0.0246	0.782
$\kappa_{12,5}$	0.	-0.013	0.0193	0.500
$\kappa_{13,0}$	0.	-0.504	0.365	0.168
$\kappa_{13,1}$	0.	0.322	0.1694	0.057
$\kappa_{13,2}$	0.	0.06	0.2009	0.766
$\kappa_{13,3}$	0.	-0.036	0.0264	0.172
$\kappa_{13,4}$	0.	0.005	0.0361	0.893
$\kappa_{13,5}$	0.	-0.032	0.0254	0.202
$\kappa_{23,0}$	0.	0.145	0.3721	0.697
$\kappa_{23,1}$	0.	0.133	0.1629	0.414
$\kappa_{23,2}$	0.	-0.194	0.2	0.331
$\kappa_{23,3}$	0.	-0.02	0.0248	0.419
$\kappa_{23,4}$	0.	0.041	0.0366	0.262
$\kappa_{23,5}$	0.	-0.015	0.025	0.551
$\kappa_{123,0}$	1.	1.188	0.9986	0.235
$\kappa_{123,1}$	0.	0.184	0.4234	0.664
$\kappa_{123,2}$	0.	-0.541	0.4124	0.190
$\kappa_{123,3}$	0.5	0.608	0.4525	0.179
$\kappa_{123,4}$	0.	-0.011	0.0324	0.729
$\kappa_{123,5}$	0.	0.049	0.031	0.113
$\kappa_{123,6}$	0.	0.003	0.0411	0.947
$\kappa_{123,7}$	-0.2	-0.212	0.0351	<0.001

Table 5.4: Estimated parameters of final model for Scenario 2

Parameter	True Value	Estimated Value	Standard Error	p-value
β_0	4.	4.034	0.0569	<0.001
β_1	-1.	-1.017	0.0231	<0.001
β_2	0.693	0.727	0.0621	<0.001
β_3	0.02	-0.017	0.0314	0.596
β_4	1.099	1.138	0.0469	<0.001
β_5	0.005	-0.03	0.0343	0.389
$\kappa_{12,0}$	0.	0.007	0.0449	0.871
$\kappa_{13,0}$	0.	-0.297	0.1609	0.065
$\kappa_{13,1}$	0.	0.23	0.1012	0.023
$\kappa_{13,3}$	0.	-0.04	0.0157	0.011
$\kappa_{23,0}$	0.	0.317	0.1895	0.095
$\kappa_{23,2}$	0.	-0.245	0.1243	0.049
$\kappa_{23,4}$	0.	0.041	0.0217	0.060
$\kappa_{123,0}$	1.	0.45	0.493	0.361
$\kappa_{123,1}$	0.	0.227	0.1182	0.055
$\kappa_{123,2}$	0.	-0.155	0.1948	0.425
$\kappa_{123,3}$	0.5	0.726	0.1371	<0.001
$\kappa_{123,5}$	0.	0.027	0.0137	0.047
$\kappa_{123,7}$	-0.2	-0.242	0.0272	<0.001

Figure 5.2 shows the results for the second constructed data set. Panels A through C of the figure show the fitted log dose-response curves for each individual drug, used alone. The log-dose response curves were used to construct initial estimates for the extended Kong and Lee parametric model.

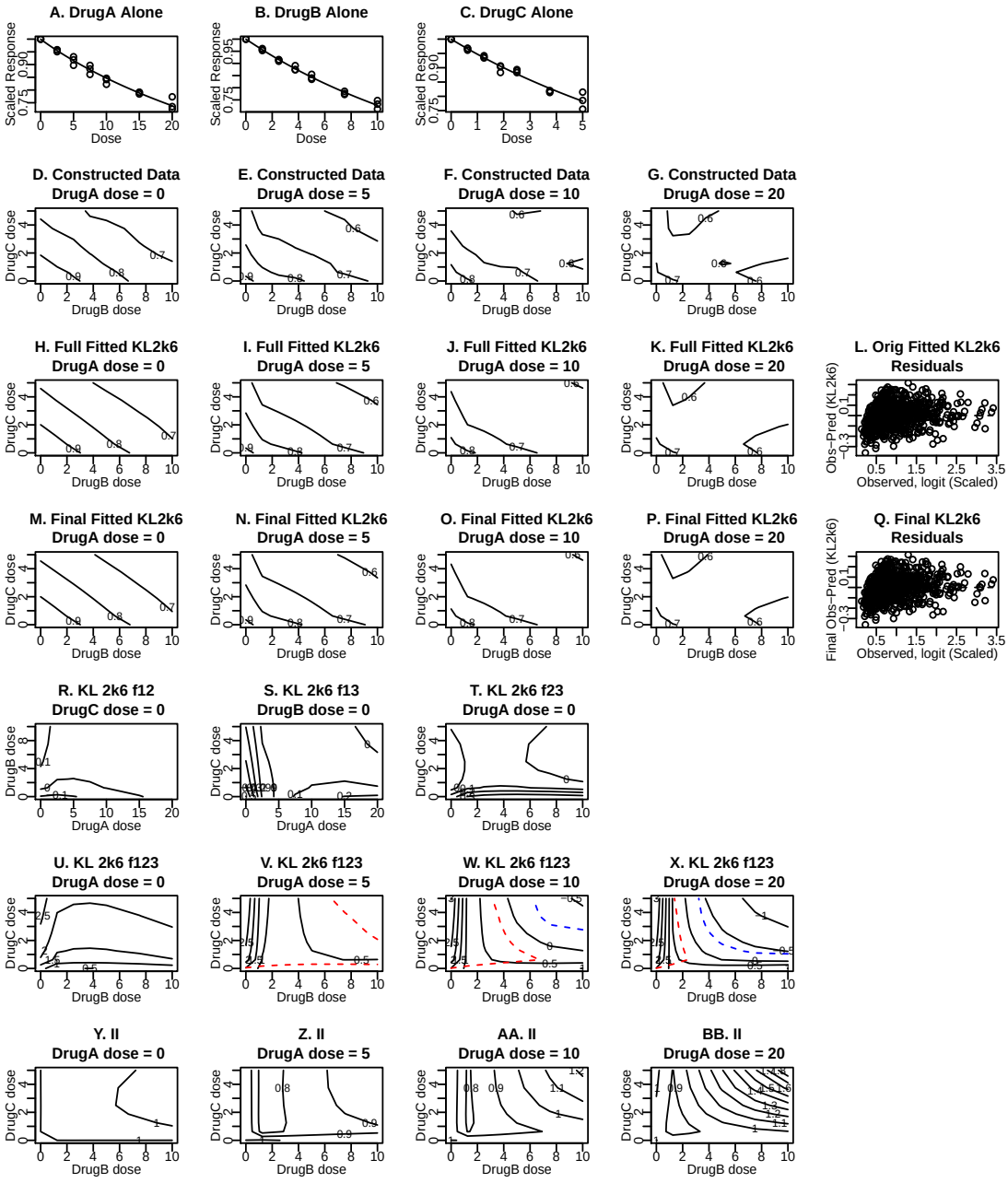

Figure 5.2: Results from second constructed data set, with local 3-way synergy in one region and local 3-way antagonism in another.

Panels D through G in the figure show contour plots of the "observed" constructed data. The contour plots of the "observed" data in Panels E through G have some lines curved downward, but other lines that are not curved downward, particularly at the highest dose, reflecting the mix of local 3-way synergy and antagonism between Drug A, Drug B and Drug C. The lines in Panel D are slightly jagged, but more straight, reflecting the fact that this synergy and antagonism is 3-way synergy and antagonism, and is not present when Drug A is not present.

Panels H through K show contour plots of the full fitted model. The fitted surfaces match the observed surfaces fairly well, curved downward in some regions, but not in others. Panel L shows the residuals of the full fitted model, plotted against the observed (logit) response.

Panels M through P show contour plots of the final fitted model. There appears to be little difference between the plots of the final model and the full model. Panel Q shows the residuals of the final fitted model, which do not appear to be very different from those of the full fitted model, shown in Panel L.

Panels R, S and T respectively show contour plots of the f_{12}, f_{13} and f_{23} functions of the full fitted model. Ideally these plots would be perfectly flat on the zero plane, reflecting the true value zero of the functions. Although both positive and negative contour lines of the surface indicate that synergy or antagonism may be present, the lack of dashed red lines and dashed blue lines indicates that it is not statistically significant at any dose combination.

Panels U through X show contour plots of the f_{123} function of the full fitted model. The positive contour lines indicate regions where synergy is present, while the negative contour lines indicate regions where antagonism is present. The dashed red lines and dashed blue lines respectively indicate regions where the synergy and antagonism is statistically significant.

Panels Y through BB show contour plots of the Interaction Index based on the full fitted model. The contour lines less than 1 indicate the dose combinations where synergy is present, while the contour lines greater than 1 indicate the dose combinations where antagonism is present.

5.4 Model Evaluation Using Simulated Data

An R program was created to implement and evaluate the goodness of fit of the extended Kong and Lee parametric method for 3 drugs described in Section 5.2. The method was evaluated using simulated data under two different synergy scenarios, the same scenarios used to evaluate the response surface estimation of the method in Section 5.3: 2-way synergy between one pair of drugs combined with 3-way synergy between all of the drugs, and a combination of localized 3-way synergy and 3-way antagonism.

The goodness of fit of the extended Kong and Lee parametric method was evaluated using simulated data constructed as described in the Section 5.3.1. For each of the two synergy scenarios described, 100 simulated data sets were constructed, and each simulated data set was analyzed using the extended Kong and Lee parametric method. Both the full model and the final model, selected by the model selection algorithm, were evaluated.

The goodness of fit was evaluated in two ways: by evaluating the estimated parameters of the model, and by evaluating the estimated functions of the model, f_{12}, f_{13}, f_{23} and f_{123}, at each dose combination; this second evaluation included an evaluation of the classification of the dose combination as either synergistic, antagonistic or additive.

5.4.1 Goodness of Fit Based On Estimated Parameters

To evaluate the goodness of fit of the model, each parameter was evaluated as follows. First, when evaluating a final model, the percentage of time that the parameter was included in the final model was calculated (this evaluation was skipped for the full models). Next the mean of the estimated parameter was calculated; if the parameter was not included in the model for a given simulation run, its estimate was taken as zero for that simulation run, so the mean is based on all 100 simulation runs. Then the mean of the standard error of the parameters estimates was calculated; this mean was only based on the simulation runs where the parameter was actually included in the model. Next, the coverage of the parameter's 95% confidence interval was calculated, by calculating the percentage of time the estimated confidence interval contained the

true value of the parameter. If the parameter was not included in the model for a given simulation run, and its true value was zero, then the confidence interval was considered to have included the true value. If the parameter was not included in the model for a given simulation run, and its true value was not zero, then the confidence interval was considered to have not included the true value. The mean bias of each estimate was calculated as the mean of the difference between each estimate and the true value. The percent bias was calculated as the bias divided by the true value, times 100%; the percent bias was not calculated in cases where the true value was zero.

Table 5.5 shows the results of the full model for the first scenario, 2-way synergy between one pair of drugs combined with 3-way synergy. As the table shows, most of the parameters have a very small percent bias, except for the β_3 and β_5 parameters, which have very small true values. The coverage of the confidence intervals is fairly high, and close to the nominal 95% for most parameters.

Table 5.6 shows the results of the final model for first scenario. With the final model, the relative bias of $\kappa_{123,7}$, one of the two non-zero κ parameters, is much higher than in the full model. The coverage for the confidence intervals is lower, as low as 57% for some cases including $\kappa_{123,7}$.

Based on these results, it appears that there may be a problem with the model selection algorithm, and the full model may be a better model to use. The similarity of the f_{12}, f_{13}, f_{23} and f_{123} functions may make it difficult to distinguish the parameters from each other, and difficult to distinguish two-way synergy from three-way synergy. Although there is no 2-way synergy or antagonism between Drug 1 and Drug 3, and between Drug 2 and Drug 3, a number of κ parameters from the f_{13} and f_{23} functions, which model those 2-way interactions, are being included in the final model approximately 50% of the time, even though all of those parameters have a true value of zero. And perhaps not coincidentally, $\kappa_{123,7}$, which has a true value that is non-zero, and which is a parameter of f_{123} that models the three-way synergy, is being excluded from the final model nearly 25% of the time.

Table 5.7 shows the results of the full model for the second scenario, local 3-way synergy and antagonism between all three drugs. As the table shows, most of the

Table 5.5: Evaluation of estimated parameters of full model of Scenario 1

Parameter	True Value	Mean Estimate	Mean Standard Error	Confidence Interval Coverage	Bias	Percent Bias
β_0	4.	3.998	0.0727	96%	-0.002	-0.0%
β_1	-1.	-1.001	0.0326	97%	-0.001	0.1%
β_2	0.693	0.686	0.0731	95%	-0.007	-1.0%
β_3	0.02	0.021	0.0461	99%	0.001	2.8%
β_4	1.099	1.093	0.0524	97%	-0.005	-0.5%
β_5	0.005	0.008	0.0461	96%	0.003	60.0%
$\kappa_{12,0}$	0.4	0.407	0.3842	97%	0.007	1.9%
$\kappa_{12,1}$	0.	-0.003	0.1821	94%	-0.003	NA
$\kappa_{12,2}$	0.	-0.002	0.1752	98%	-0.002	NA
$\kappa_{12,3}$	0.	0.001	0.0284	97%	0.001	NA
$\kappa_{12,4}$	0.	0.001	0.0268	94%	0.001	NA
$\kappa_{12,5}$	0.	-0.001	0.0208	96%	-0.001	NA
$\kappa_{13,0}$	0.	0.046	0.3426	95%	0.046	NA
$\kappa_{13,1}$	0.	-0.013	0.159	94%	-0.013	NA
$\kappa_{13,2}$	0.	-0.024	0.1872	94%	-0.024	NA
$\kappa_{13,3}$	0.	0.001	0.0251	94%	0.001	NA
$\kappa_{13,4}$	0.	0.003	0.0334	97%	0.003	NA
$\kappa_{13,5}$	0.	0.003	0.0241	93%	0.003	NA
$\kappa_{23,0}$	0.	0.018	0.348	94%	0.018	NA
$\kappa_{23,1}$	0.	0.001	0.1535	96%	0.001	NA
$\kappa_{23,2}$	0.	-0.01	0.1875	94%	-0.01	NA
$\kappa_{23,3}$	0.	0.	0.0239	97%	0.	NA
$\kappa_{23,4}$	0.	0.001	0.0337	95%	0.001	NA
$\kappa_{23,5}$	0.	0.001	0.0239	98%	0.001	NA
$\kappa_{123,0}$	0.	-0.099	1.0736	95%	-0.099	NA
$\kappa_{123,1}$	0.	-0.001	0.4489	97%	-0.001	NA
$\kappa_{123,2}$	0.	0.02	0.4361	96%	0.02	NA
$\kappa_{123,3}$	0.	0.052	0.4557	95%	0.052	NA
$\kappa_{123,4}$	0.	0.003	0.0342	96%	0.003	NA
$\kappa_{123,5}$	0.	0.001	0.0324	97%	0.001	NA
$\kappa_{123,6}$	0.	-0.002	0.0406	95%	-0.002	NA
$\kappa_{123,7}$	0.08	0.072	0.0436	95%	-0.008	-9.9%

Table 5.6: Evaluation of estimated parameters of final model of Scenario 1

Parameter	True Value	Included In Final Model	Mean Estimate	Mean Standard Error	Confidence Interval Coverage	Bias	Percent Bias
β_0	4.	100%	3.999	0.0579	90%	-0.001	-0.0%
β_1	-1.	100%	-1.001	0.0239	89%	-0.001	0.1%
β_2	0.693	100%	0.683	0.0626	94%	-0.011	-1.5%
β_3	0.02	100%	0.024	0.0347	90%	0.004	20.0%
β_4	1.099	100%	1.093	0.0473	95%	-0.006	-0.5%
β_5	0.005	100%	0.009	0.0357	92%	0.004	88.8%
$\kappa_{12,0}$	0.4	100%	0.426	0.1456	81%	0.026	6.5%
$\kappa_{12,1}$	0.	51%	-0.01	0.0797	77%	-0.01	NA
$\kappa_{12,2}$	0.	47%	-0.005	0.0838	84%	-0.005	NA
$\kappa_{12,3}$	0.	19%	0.001	0.0197	91%	0.001	NA
$\kappa_{12,4}$	0.	19%	0.	0.0194	95%	0.	NA
$\kappa_{12,5}$	0.	21%	0.001	0.0173	89%	0.001	NA
$\kappa_{13,0}$	0.	100%	0.04	0.1416	72%	0.04	NA
$\kappa_{13,1}$	0.	54%	-0.005	0.0883	72%	-0.005	NA
$\kappa_{13,2}$	0.	48%	-0.027	0.0892	81%	-0.027	NA
$\kappa_{13,3}$	0.	25%	-0.001	0.0198	85%	-0.001	NA
$\kappa_{13,4}$	0.	13%	0.003	0.0293	94%	0.003	NA
$\kappa_{13,5}$	0.	24%	0.004	0.0213	88%	0.004	NA
$\kappa_{23,0}$	0.	100%	0.041	0.1434	80%	0.041	NA
$\kappa_{23,1}$	0.	46%	-0.011	0.0769	83%	-0.011	NA
$\kappa_{23,2}$	0.	48%	-0.009	0.0961	77%	-0.009	NA
$\kappa_{23,3}$	0.	17%	0.	0.019	95%	0.	NA
$\kappa_{23,4}$	0.	19%	-0.001	0.0274	95%	-0.001	NA
$\kappa_{23,5}$	0.	21%	0.004	0.0203	91%	0.004	NA
$\kappa_{123,0}$	0.	100%	-0.163	0.5187	75%	-0.163	NA
$\kappa_{123,1}$	0.	97%	0.016	0.1515	71%	0.016	NA
$\kappa_{123,2}$	0.	99%	0.042	0.149	76%	0.042	NA
$\kappa_{123,3}$	0.	99%	0.055	0.1686	74%	0.055	NA
$\kappa_{123,4}$	0.	13%	0.003	0.0242	98%	0.003	NA
$\kappa_{123,5}$	0.	15%	0.002	0.021	94%	0.002	NA
$\kappa_{123,6}$	0.	20%	0.	0.0299	89%	0.	NA
$\kappa_{123,7}$	0.08	76%	0.065	0.0287	73%	-0.015	-18.3%

parameters have a very small percent bias, except for the β_3 and β_5 parameters, which have very small true values, so even a small bias becomes a very large percent bias. The coverage of the confidence intervals is fairly high, at least 95% for most parameters.

Table 5.7: Evaluation of estimated parameters of full model of Scenario 2

Parameter	True Value	Mean Estimate	Mean Standard Error	Confidence Interval Coverage	Bias	Percent Bias
β_0	4.	4.001	0.0718	97%	0.001	0.0%
β_1	-1.	-1.002	0.0321	97%	-0.002	0.2%
β_2	0.693	0.689	0.0722	95%	-0.004	-0.6%
β_3	0.02	0.018	0.0453	97%	-0.002	-7.9%
β_4	1.099	1.094	0.052	97%	-0.004	-0.4%
β_5	0.005	0.007	0.0454	97%	0.002	37.9%
$\kappa_{12,0}$	0.	0.001	0.321	98%	0.001	NA
$\kappa_{12,1}$	0.	-0.002	0.1535	95%	-0.002	NA
$\kappa_{12,2}$	0.	0.001	0.1481	98%	0.001	NA
$\kappa_{12,3}$	0.	0.	0.0242	96%	0.	NA
$\kappa_{12,4}$	0.	0.001	0.0231	95%	0.001	NA
$\kappa_{12,5}$	0.	-0.001	0.0178	97%	-0.001	NA
$\kappa_{13,0}$	0.	0.043	0.3354	96%	0.043	NA
$\kappa_{13,1}$	0.	-0.011	0.1558	94%	-0.011	NA
$\kappa_{13,2}$	0.	-0.023	0.1842	94%	-0.023	NA
$\kappa_{13,3}$	0.	0.001	0.0247	94%	0.001	NA
$\kappa_{13,4}$	0.	0.003	0.0331	97%	0.003	NA
$\kappa_{13,5}$	0.	0.003	0.0227	93%	0.003	NA
$\kappa_{23,0}$	0.	0.012	0.3393	94%	0.012	NA
$\kappa_{23,1}$	0.	0.002	0.1507	97%	0.002	NA
$\kappa_{23,2}$	0.	-0.008	0.1843	94%	-0.008	NA
$\kappa_{23,3}$	0.	0.	0.0237	96%	0.	NA
$\kappa_{23,4}$	0.	0.001	0.0333	94%	0.001	NA
$\kappa_{23,5}$	0.	0.001	0.0224	98%	0.001	NA
$\kappa_{123,0}$	1.	0.941	0.9409	96%	-0.059	-5.9%
$\kappa_{123,1}$	0.	-0.005	0.3979	97%	-0.005	NA
$\kappa_{123,2}$	0.	0.006	0.3865	96%	0.006	NA
$\kappa_{123,3}$	0.5	0.54	0.4167	96%	0.04	8.0%
$\kappa_{123,4}$	0.	0.002	0.03	97%	0.002	NA
$\kappa_{123,5}$	0.	0.	0.0297	95%	0.	NA
$\kappa_{123,6}$	0.	-0.002	0.0381	94%	-0.002	NA
$\kappa_{123,7}$	-0.2	-0.205	0.0325	95%	-0.005	2.7%

Table 5.8 shows the results of the final model for second scenario. With the final model, the relative bias of $\kappa_{123,3}$, one of the three non-zero κ parameters, is much higher than in the full model. The coverage for the confidence intervals is lower, as low as 82% for some cases including $\kappa_{123,3}$.

As with final model results for the first scenario, it appears that there may be

a problem with the model selection algorithm, and the full model may be a better model to use. In this scenario there is no 2-way synergy or antagonism, so all of the κ parameters of the f_{12}, f_{13} and f_{23} functions have a true value of zero, and none of them should be included in the final model (except the "intercepts", $\kappa_{12,0}$, $\kappa_{13,0}$, and $\kappa_{23,0}$ which are always included in the model). But, some of those κ parameters are being included in the final model over 40% of the time.

Table 5.8: Evaluation of estimated parameters of final model of Scenario 2

Parameter	True Value	Included In Final Model	Mean Estimate	Mean Standard Error	Confidence Interval Coverage	Bias	Percent Bias
β_0	4.	100%	4.004	0.0556	94%	0.004	0.1%
β_1	-1.	100%	-1.003	0.0225	93%	-0.003	0.3%
β_2	0.693	100%	0.69	0.0598	94%	-0.003	-0.4%
β_3	0.02	100%	0.019	0.0324	93%	-0.001	-7.3%
β_4	1.099	100%	1.097	0.0457	93%	-0.002	-0.2%
β_5	0.005	100%	0.004	0.0338	91%	-0.001	-12.4%
$\kappa_{12,0}$	0.	100%	-0.002	0.1225	84%	-0.002	NA
$\kappa_{12,1}$	0.	44%	0.003	0.068	81%	0.003	NA
$\kappa_{12,2}$	0.	48%	0.001	0.0799	87%	0.001	NA
$\kappa_{12,3}$	0.	15%	-0.001	0.017	95%	-0.001	NA
$\kappa_{12,4}$	0.	24%	0.	0.017	93%	0.	NA
$\kappa_{12,5}$	0.	19%	-0.001	0.0157	94%	-0.001	NA
$\kappa_{13,0}$	0.	100%	0.039	0.1352	75%	0.039	NA
$\kappa_{13,1}$	0.	50%	-0.013	0.0831	76%	-0.013	NA
$\kappa_{13,2}$	0.	47%	-0.017	0.0877	83%	-0.017	NA
$\kappa_{13,3}$	0.	23%	0.001	0.0185	86%	0.001	NA
$\kappa_{13,4}$	0.	13%	0.002	0.0291	94%	0.002	NA
$\kappa_{13,5}$	0.	22%	0.002	0.0203	91%	0.002	NA
$\kappa_{23,0}$	0.	100%	0.008	0.1287	84%	0.008	NA
$\kappa_{23,1}$	0.	40%	-0.001	0.0773	92%	-0.001	NA
$\kappa_{23,2}$	0.	41%	-0.003	0.0929	86%	-0.003	NA
$\kappa_{23,3}$	0.	17%	0.	0.0185	96%	0.	NA
$\kappa_{23,4}$	0.	16%	0.	0.0281	94%	0.	NA
$\kappa_{23,5}$	0.	13%	0.001	0.0203	97%	0.001	NA
$\kappa_{123,0}$	1.	100%	0.94	0.5091	82%	-0.06	-6.0%
$\kappa_{123,1}$	0.	100%	0.001	0.1398	80%	0.001	NA
$\kappa_{123,2}$	0.	100%	0.008	0.1423	86%	0.008	NA
$\kappa_{123,3}$	0.5	100%	0.527	0.1674	83%	0.027	5.4%
$\kappa_{123,4}$	0.	13%	0.002	0.0199	96%	0.002	NA
$\kappa_{123,5}$	0.	20%	0.001	0.0183	92%	0.001	NA
$\kappa_{123,6}$	0.	24%	-0.002	0.0258	91%	-0.002	NA
$\kappa_{123,7}$	-0.2	100%	-0.204	0.0253	88%	-0.004	2.2%

5.4.2 Goodness of Fit Based On Model Functions

The second way of evaluating the goodness of fit is to evaluate the values of the f_{12}, f_{13}, f_{23} and f_{123} functions at each combination of doses, and to evaluate the classification of the dose as either synergistic, antagonistic or additive. Each function was evaluated at each dose combination as follows.

First, the mean of the function's estimated value from the simulation runs was calculated. The bias was calculated as the difference between the mean estimated value and the true value of the function. The percent bias was calculated as the bias divided by the true value of the function, times 100%; if the true value of the function is zero, then the percent bias cannot be calculated.

The mean square error of the estimates was calculated as the mean of the square of the difference between the estimated value of the function for a given simulation run and its true value.

The confidence interval coverage was calculated as the percentage of times that the estimated 95% confidence interval for the function included its true value.

The last method of evaluating the goodness of fit of the model functions was based on the classification of each dose combination as either synergistic, antagonistic or additive. The classification of a dose combination is determined from the estimated value of the function at that dose combination, and its estimated 95% confidence interval. If the upper limit of the confidence interval is less than zero, then the dose combination is classified as synergistic; if the lower limit of the confidence interval is greater than zero, then the dose combination is classified as antagonistic; if the confidence interval includes zero, then the dose combination is classified as additive. To evaluate the goodness of fit, at each dose the number of simulation runs that were classified into each category were counted, and the percentage of runs that chose the "correct" category was recorded.

Because the classification is based on the confidence interval for the estimated value of the function, and thus its statistical significance, the "correct" category for a dose combination is not simply based on the true value of the function for that dose combination. Although negative values indicate antagonism, negative values close to zero

may not be far enough from zero to indicate significant antagonism. Similarly, although positive values indicate synergy, positive values close to zero may not be far enough from zero to indicate significant synergy. So the "correct" category must take into account not just the true value of the function, but also the "expected" width of the confidence interval for the estimate of the function.

The estimated confidence interval for the function is estimated using the delta method and the estimated standard errors for the parameters, as described in Section 5.2.2. The standard errors for the parameters are estimated as part of the nonlinear regression performed on the model of the extended Kong and Lee parametric method. The nonlinear regression software used in this implementation of the extended Kong and Lee parametric method, the nls() function in R, uses the Gauss-Newton method to estimate the regression parameters and their standard errors. The estimated standard errors are based on the design of the model and the residual mean square error, which estimates the standard deviation.

In the simulations used to evaluate the goodness of fit, the true standard deviation is known, and used to create the simulated data. In the same way that the Gauss-Newton method uses the estimated standard deviation to estimate the standard errors of the estimated parameters in the model, the known true standard deviation can be used to calculate a "true standard error" for each known, true parameter. The delta method can then be used to calculate the "true standard errors" of the model functions f_{12}, f_{13}, f_{23} and f_{123}. The "true standard errors" of the model functions can be used to calculate confidence intervals for the model functions. Finally, these confidence intervals can then be used to define the "correct" category for a dose combination.

The "true standard error" for each regression parameter was calculated from the known true standard deviation and a design matrix based on derivatives of the model specified in Equation (5.3). See Appendix A for details.

The "true standard errors" for the regression parameters were then used to calculate the "true standard error" for each model function, using the Delta method, in the same way the estimated standard error for the model function is calculated, as described in Section 5.2.2. The "true standard error" for the model function was used to calculate

a confidence interval for the true value of the function. The confidence interval based on the "true standard error" was then used to determine the "correct" category for each dose combination. If the confidence interval was completely above zero, then the correct category was defined to be synergism. If the confidence interval was completely below zero, then the correct category was defined to be antagonism. If the confidence interval included zero, then the correct category was defined to be additive.

Because of the large number of dose combinations, especially for f_{123}, it is generally more useful to examine summaries of the evaluations measures, rather than the individual measures at particular dose combinations. The evaluation measures are summarized graphically below, but tables listing the measures at each dose combination are included in Appendix B

Figure 5.3 shows box plots summarizing the results for function f_{12}. The figure shows the results for percent bias, bias, confidence interval coverage, and correct classification for both models of both scenarios.

The percent bias and bias are both fairly small, and close to zero (for the second scenario, the true value of f_{12} is zero, so the percent bias could not be calculated). For both scenarios, the full model generally performs better than the final model, which has a noticeable negative bias.

For the full model of both scenarios, the confidence interval coverage is very close to its nominal level of 95%. The coverage is somewhat less for the final model of both scenarios, suggesting that the final model is underestimating the variability in the model.

The percentage of correct classification is very good for both models of both scenarios.

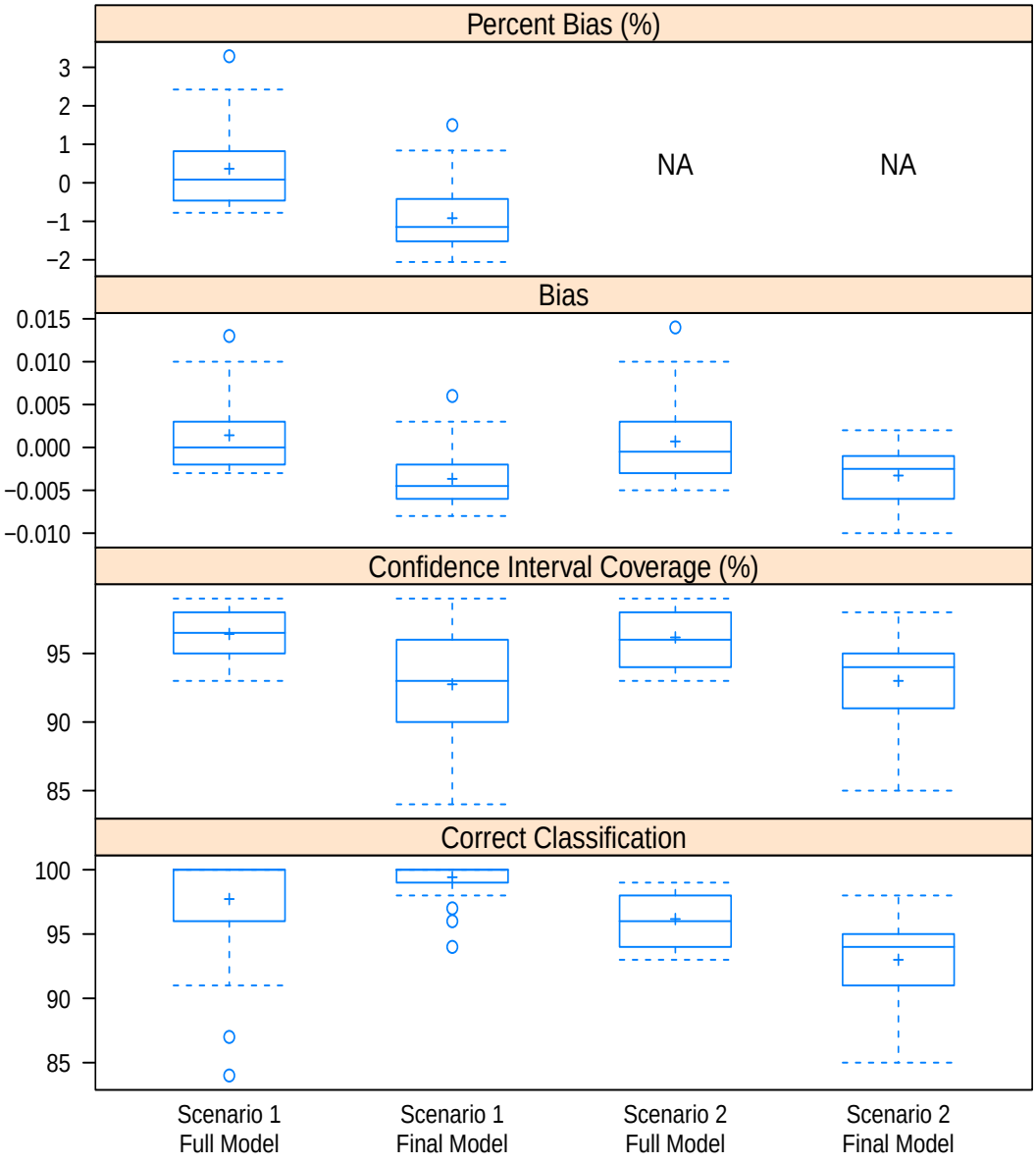

Figure 5.3: Goodness of fit evaluation of function f_{12}.

Figure 5.4 shows box plots summarizing the results for function f_{13}. The true value of f_{13} is zero for both scenarios, so percent bias could not be calculated for either of them. The bias is similar for both models of both scenarios, and all models underestimated the true value of f_{13}.

For the confidence interval coverage and correct classification, the full model performs better than the final model for both scenarios, although the confidence interval coverage does not quite reach the nominal 95% level of the confidence interval.

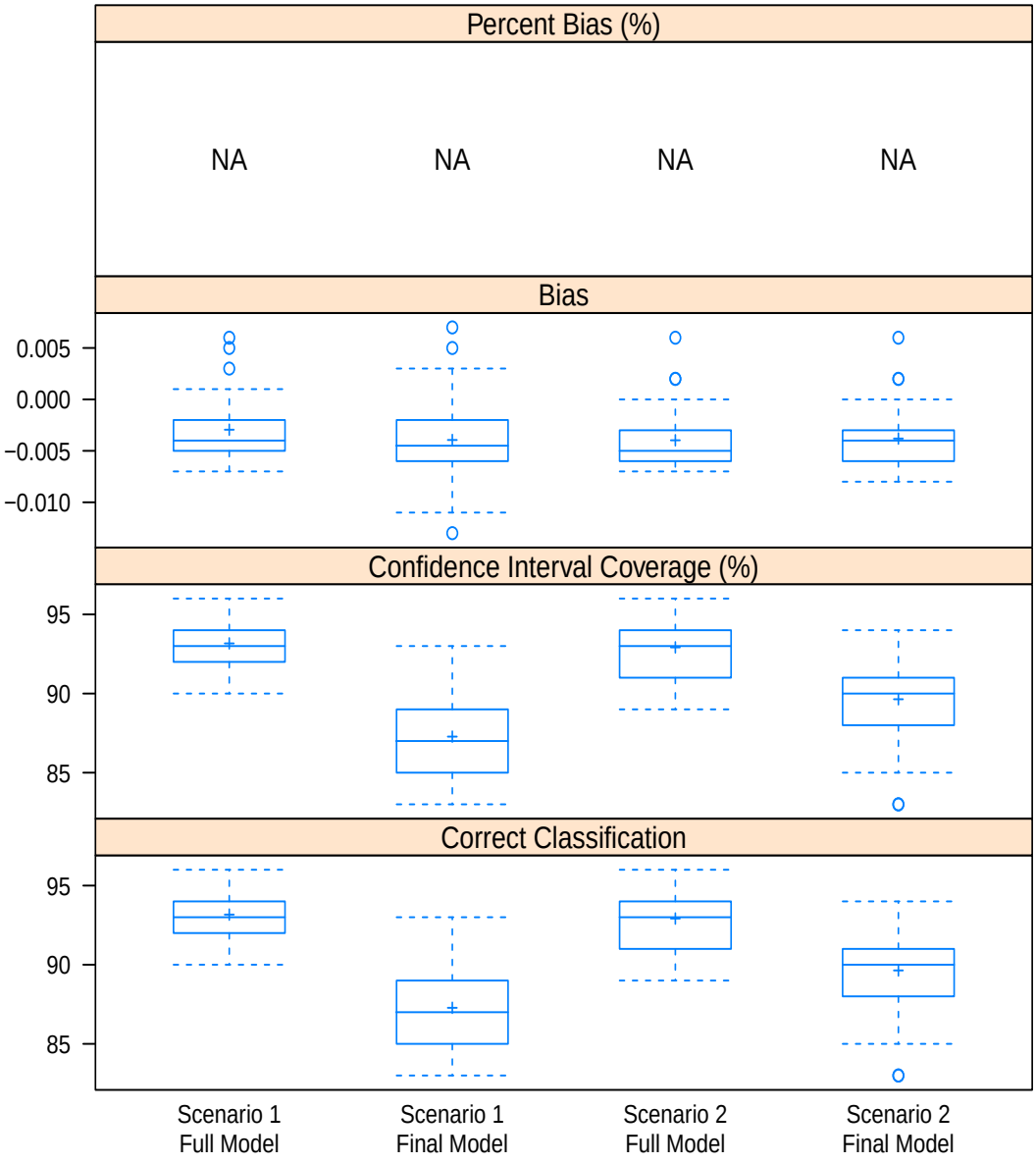

Figure 5.4: Goodness of fit evaluation of function f_{13}.

Figure 5.5 shows box plots summarizing the results for function f_{23}. As with f_{13}, The true value of f_{23} is zero for both scenarios, so percent bias could not be calculated for either of them. The bias is similar for both models of both scenarios, and all models overestimated the true value of f_{23}.

For the confidence interval coverage and correct classification, the full model again performs better than the final model for both scenarios, and the confidence interval coverage is very close to the 95% level of the confidence interval.

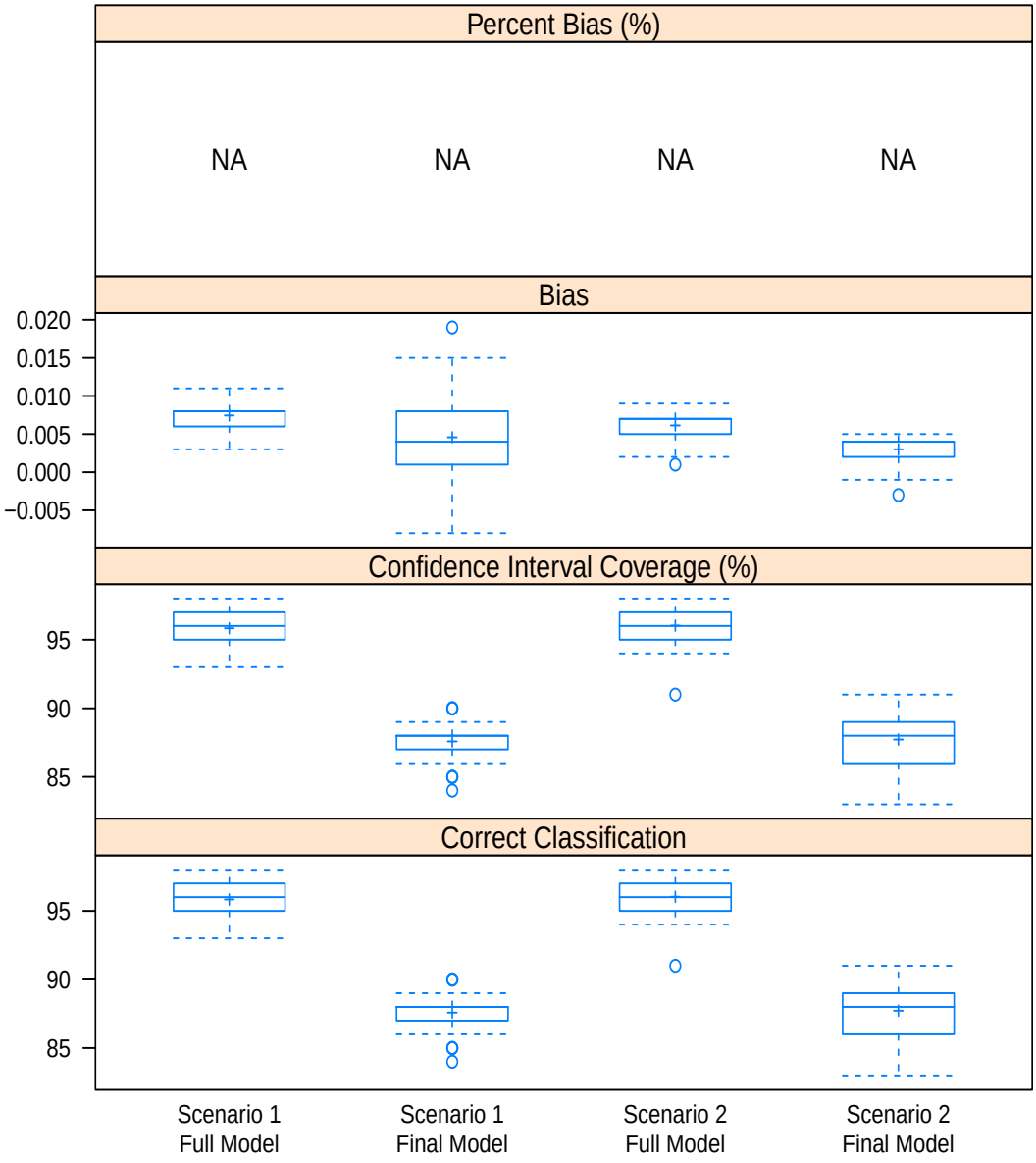

Figure 5.5: Goodness of fit evaluation of function f_{23}.

For function f_{123}, most of the percent bias measurements are between -15% and 15%, but there are a few percent bias measurements that are quite extreme, on the order of 400% and -200%. If the extreme measurements are included in a graph, then the bulk of the measurements are indistinguishable from each other because the scale has to be so large to accommodate the extreme measurements.

Figure 5.6 shows box plots summarizing the results for function f_{123}, excluding all percent bias measurements outside the range of -15% to 15%, which are not shown in the plot. A total of 11 large measurements were removed, and these large measurements will be discussed shortly. The remaining measurements are fairly small, and generally within 5%.

Under both scenarios there was little difference between the percent bias and the bias of the full model and the final model. The confidence interval coverage of the full model was much closer than the final model to the nominal 95% level of the confidence interval. The final model performed more accurate classification than the full model for the first scenario, but the classification by the full model was still very accurate.

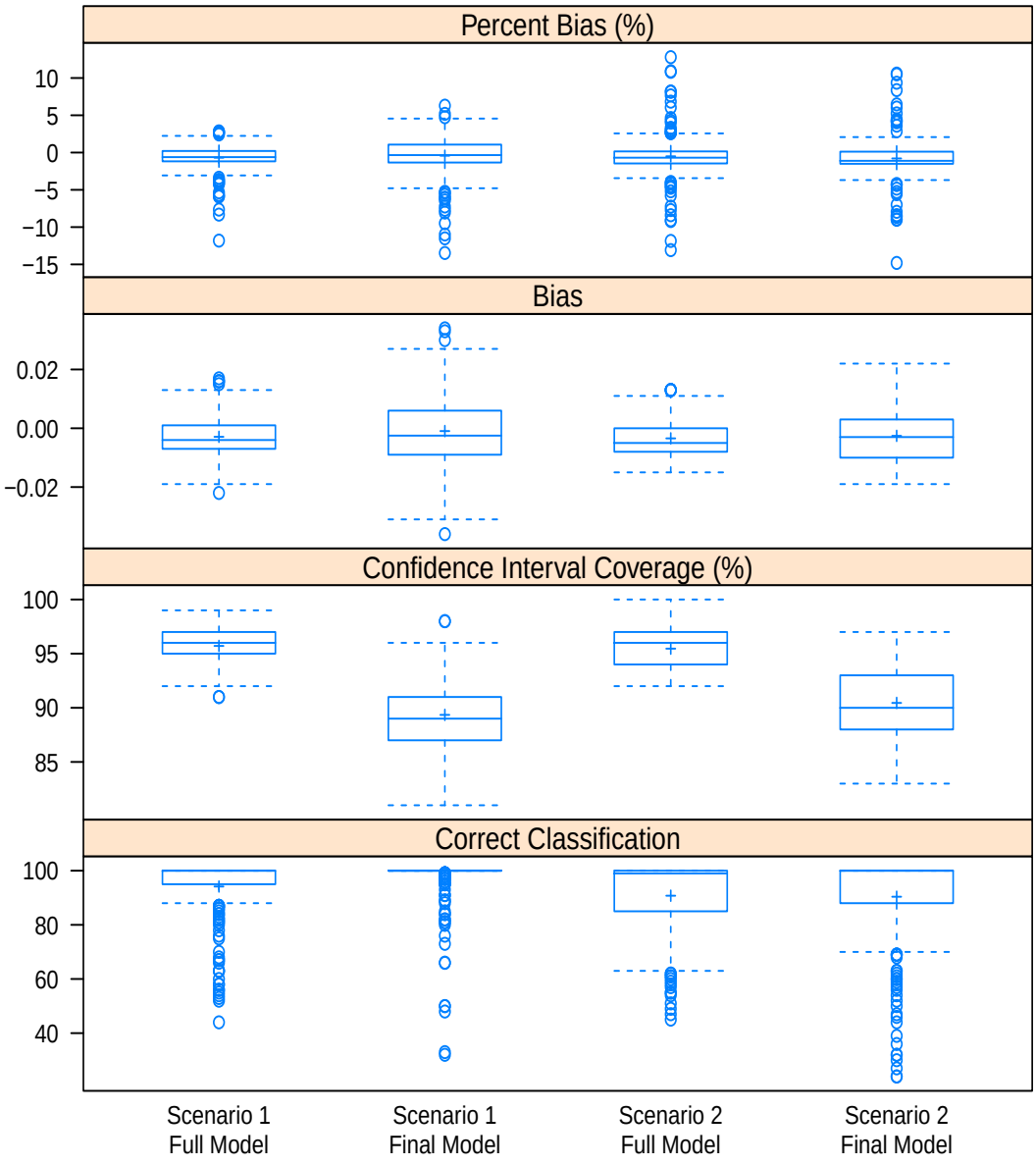

Figure 5.6: Goodness of fit evaluation of function f_{123}. Some extreme percent bias measurements have been excluded; see text for details.

In Figure 5.6, eleven extremely large percent bias measurements from the full and final models for Scenario 2 were excluded from the plot to make it more readable. As described previously, the percent bias is calculated as the bias divided by the true value of f_{123}, multiplied by 100%. But these extreme percent bias measurements were not due to extreme bias measurements. Instead, they were caused by dose combinations where the bias of the estimate was not unusual, but the true value of f_{123} at that dose combination was so close to zero that the percent bias became huge. Figure 5.7 shows the percent bias measurements from Figure 5.6, plotted against their corresponding true value of f_{123}. As the figure shows, the extreme percent bias measurements for Scenario 2 all occurred where the true value of f_{123} was very close to zero.

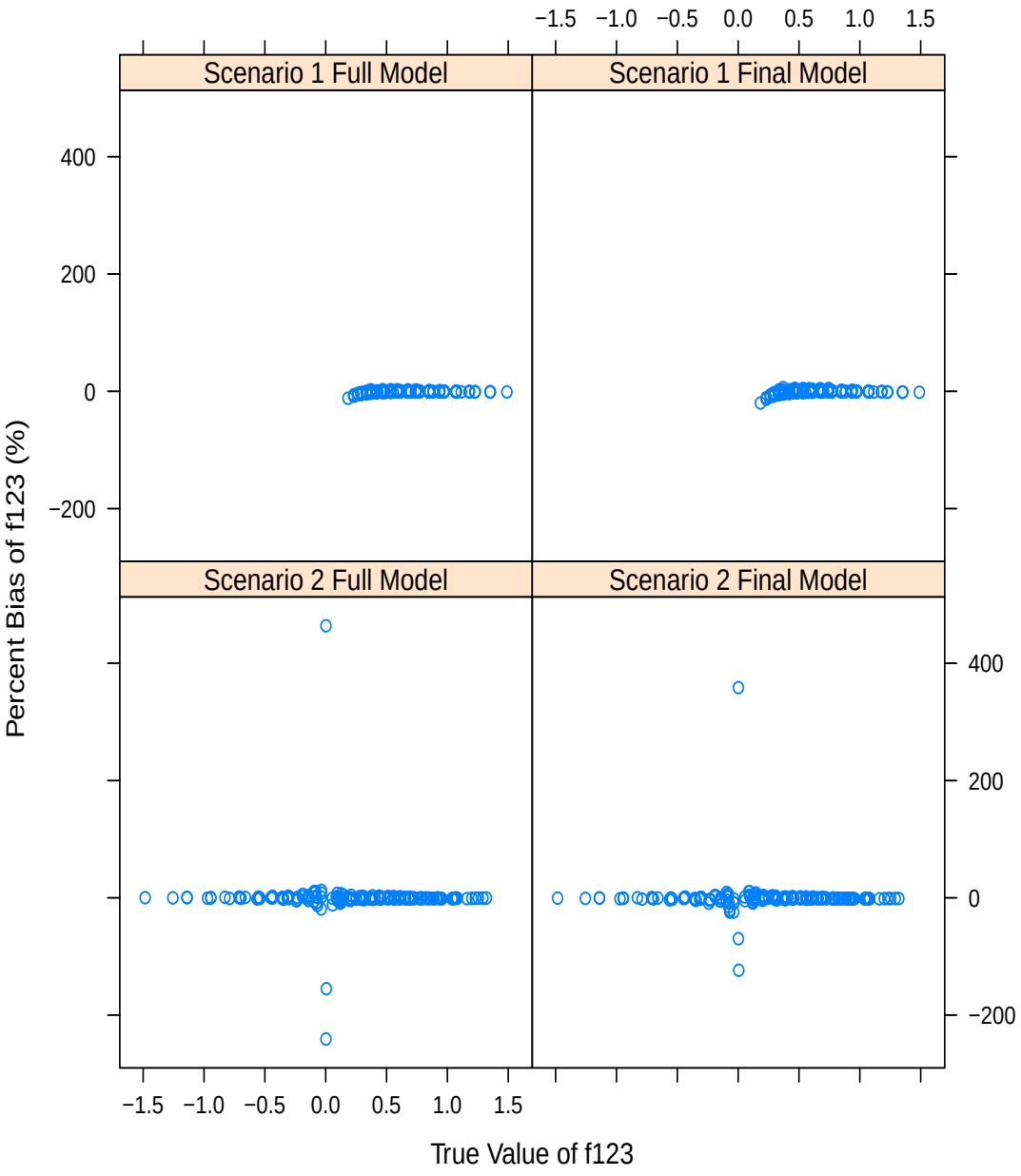

Figure 5.7: Goodness of fit evaluation of function f_{123}.

Chapter 6

Extend Kong and Lee's Semiparametric Model to Three or More Drugs

6.1 General Model Description

The next synergy method that this thesis extends to handle three or more drugs is Kong and Lee's semiparametric method, which was described in Section 2.5.

As with Kong and Lee's semiparametric method, we use the Loewe additivity model as a starting point, although now that there are n drugs we use the extended Loewe additivity model in (3.3). Assume that function $F_j(D_j)$ denotes the dose-response curve for drug j alone, for $1 \leq j \leq n$. Given the inverses of the dose-response functions, $F_j^{-1}(y)$, the predicted additive effect y for a combination of n doses can be calculated by replacing each $D_{y,j}$ in (3.3) by $F_j^{-1}(y)$ and then solving equation (3.3). We will denote the predicted additive effect for combination dose $(d_1, \ldots, d_n)$ as $F_0(d_1, \ldots, d_n)$.

As in Kong and Lee's method, a two-component model could be defined as:

$$Y = F_0\left(d_1, \ldots, d_n\right) + f\left(d_1, \ldots, d_n\right)$$

with $F_0\left(d_1, \ldots, d_n\right)$ modeling the theoretic additive result and $f\left(d_1, \ldots, d_n\right)$ capturing the interaction. But such a model would only capture a drug interaction compared to the individual drugs used alone, and it would capture any drug interaction, regardless of whether it was between all n drugs or only between a subset of the n drugs.

With n drugs there can be a number of different kinds of drug interactions, which will be characterized by the number of drugs involved in the interaction, and referred to as "2-way interactions", "3-way interactions", etc., all the way up to an "n-way interaction". The "2-way interactions" are between a single pair of drugs, and do not

depend in any way on the presence or absence of any of the other drugs. Similarly, "3-way interactions" are between three of the n drugs, and only depend on the presence of the three drugs involved in the interaction. The "n-way interaction" is an interaction between all n drugs, and depends on the presence of all n drugs.

With n drugs, for each of the "p-way interactions", $2 \leq p \leq n$, there are $m_p = \binom{n}{p}$ different interactions, each of which is between a unique combination of p of the n drugs. Overall, there are a total of $\sum_{p=2}^{n} m_p = M$ different interactions.

To capture all possible interactions, a $M+1$-component model will be considered:

$$Y = F_0\left(d_1, \ldots, d_n\right) + \sum_{p=2}^{n} \sum_{1 \leq i_1 < i_2 < \cdots < i_p \leq n} f_{i_1 i_2 \ldots i_p}\left(d_{i_1}, \ldots, d_{i_p}\right)$$

with conditions:

$$f_{i_1 i_2 \ldots i_p}\left(d_{i_1}, \ldots, d_{i_p}\right) = 0 \qquad \text{if } d_{i_j} = 0 \text{ for any } i_j \in \{i_1, i_2, \ldots, i_p\}.$$

F_0 is defined as above, and d_j is the amount of Drug j in the combination; d_{i_j}, where $i_j \in \{i_1, i_2, \ldots, i_p\}$, is the amount of the j^{th} drug in the combination of p drugs.

The f functions model the interactions between combinations of drugs, with the function $f_{i_1 i_2 \ldots i_p}$ modeling the p-way interaction between Drug i_1, Drug i_2, ..., and Drug i_p. For all of the f functions in the model, positive values indicate synergy, negative values indicate antagonism, and a value of zero indicates additivity, assuming a higher response is "better" (if a lower response is "better" then the interpretations of the signs are reversed).

6.2 Three Drug Model Description

To illustrate the method in greater detail, we will focus on a 3-drug model, but the concepts and implementation could be extended to the n-drug model described above.

With 3 drugs we assume that function $F_1(D_1)$ denotes the dose-response curve for drug 1 alone, $F_2(D_2)$ denotes the curve for drug 2 alone, and $F_3(D_3)$ denotes the curve for drug 3 alone. Given the inverses of the dose-response functions, $F_1^{-1}(y)$, $F_2^{-1}(y)$, and $F_3^{-1}(y)$, the predicted additive effect y for a combination of three doses can be calculated by replacing $D_{y,1}$, $D_{y,2}$, and $D_{y,3}$ in (3.2) by $F_1^{-1}(y)$, $F_2^{-1}(y)$, and $F_3^{-1}(y)$

respectively, and then solving equation (3.2). We will denote the predicted additive effect for combination dose (d_1, d_2, d_3) as $F_0(d_1, d_2, d_3)$.

To capture all possible interactions, a five-component model will be considered:

$$Y = F_0\left(d_1, d_2, d_3\right) + f_{12}\left(d_1, d_2\right) + f_{13}\left(d_1, d_3\right) + f_{23}\left(d_2, d_3\right) + f_{123}\left(d_1, d_2, d_3\right) \quad (6.1)$$

with conditions:

$$\begin{aligned} f_{12}\left(d_1, 0\right) &= f_{12}\left(0, d_2\right) = f_{13}\left(d_1, 0\right) = f_{13}\left(0, d_3\right) = f_{23}\left(d_2, 0\right) = f_{23}\left(0, d_3\right) = 0, \\ f_{123}\left(d_1, d_2, 0\right) &= f_{123}\left(d_1, 0, d_3\right) = f_{123}\left(0, d_2, d_3\right) = 0. \end{aligned}$$

where $f_{12}\left(d_1, d_2\right)$, $f_{13}\left(d_1, d_3\right)$, and $f_{23}\left(d_2, d_3\right)$ capture the two-drug interactions, and $f_{123}\left(d_1, d_2, d_3\right)$ captures the three-drug interaction.

Suppose the individual dose-effect curves are decreasing. If $f_{123}\left(d_1, d_2, d_3\right) < 0$, then the observed effect at (d_1, d_2, d_3) is more than the predicted effect based on the individual drugs and two-drug combinations, so the combination dose (d_1, d_2, d_3) is (local) synergistic. If $f_{123}\left(d_1, d_2, d_3\right) > 0$, then the observed effect at (d_1, d_2, d_3) is less than the predicted effect based on the individual drugs and two-drug combinations, so the combination dose (d_1, d_2, d_3) is (local) antagonistic. If the individual dose-effect curves were increasing, the interpretation of the sign of f_{123} would be reversed.

6.3 Estimation Method Description

As in Kong and Lee's method, the model will be fitted using a multiple step process, although now three steps will be used instead of two. The three steps can be summarized as: (1) estimate the predicted additive effect, $F_0\left(d_1, d_2, d_3\right)$, (2) estimate the two-drug interaction terms, $f_{12}\left(d_1, d_2\right)$, $f_{13}\left(d_1, d_3\right)$, and $f_{23}\left(d_2, d_3\right)$, and (3) estimate the three-drug interaction term, $f_{123}\left(d_1, d_2, d_3\right)$.

In order to estimate the predicted additive effect, $F_0\left(d_1, d_2, d_3\right)$, the individual drug dose-response curves, $F_1\left(d_1\right)$, $F_2\left(d_2\right)$ and $F_3\left(d_3\right)$, for drugs 1, 2 and 3 are first estimated using the dose-response results where each drug was used alone. This can be done either parametrically, using linear dose-response or linear log-dose response models as Kong and Lee did, or nonparametrically, using monotone smoothing based on a spline function

[18]. The estimated predicted additive effect $\widehat{F}_0(d_{1i}, d_{2i}, d_{3i})$ at the combination dose (d_{1i}, d_{2i}, d_{3i}) is then the solution of y from the Loewe's additivity model equation:

$$\frac{d_{1i}}{\widehat{F}_1^{-1}(y)} + \frac{d_{2i}}{\widehat{F}_2^{-1}(y)} + \frac{d_{3i}}{\widehat{F}_3^{-1}(y)} = 1.$$

Once the predicted additive effect, $F_0(d_1, d_2, d_3)$, has been estimated, the two-drug interaction terms, $f_{23}(d_2, d_3)$, $f_{13}(d_1, d_3)$, and $f_{12}(d_1, d_2)$, can then be estimated. They are estimated using subsets of the data: the subset where $d_1 = 0$ is used to estimate $f_{23}(d_2, d_3)$, the subset where $d_2 = 0$ is used to estimate $f_{13}(d_1, d_3)$, and the subset where $d_3 = 0$ is used to estimate $f_{12}(d_1, d_2)$. After the two-drug interaction terms have been estimated, the three-drug interaction term, $f_{123}(d_1, d_2, d_3)$ is estimated.

Two different approaches were considered for estimating the two-drug interaction terms, $f_{12}(d_1, d_2)$, $f_{13}(d_1, d_3)$, and $f_{23}(d_2, d_3)$, and the three-drug interaction term, $f_{123}(d_1, d_2, d_3)$.

The first approach was based on Kong and Lee's thin plate spline smoothing method, with Kong and Lee's smoothing method used to estimate the two-drug interaction terms. Kong and Lee's smoothing method was extended to smooth thin plate splines of order 3, as described below in Section 6.3.1, and this extended method was used to estimate the three-drug interaction term.

The second approach used Generalized Cross Validation (GCV) to smooth the thin plate splines. GCV can smooth thin plate splines of any order so it was used to estimate both the two-drug interaction terms and the three-drug interaction term. An advantage of this approach is that GCV can smooth thin plate splines of any order, so it easily scales to more than 3 drugs, while the first approach would have to be further extended to handle more than 3 drugs. Section 6.3.2 describes the GCV approach in detail. An additional advantage of the GCV approach is that the GCV software is implemented in FORTRAN and is able to perform the smoothing faster. Because of its advantages, the GCV approach was used in the implementations of the extended Kong and Lee semiparametric method that were created for this thesis.

6.3.1 Extended Kong and Lee Smoothing

Similar to Kong and Lee's approach for estimating the two-drug interaction terms, the three-drug interaction term can be estimated by minimizing a penalized residual sum of squares:

$$PRSS = \sum_{i=1}^{n} \left(w_i - f_{123}\left(d_{1i}, d_{2i}, d_{3i}\right)\right)^2 + \lambda J\left(f_{123}\right), \tag{6.2}$$

where

$$\begin{aligned} w_i &= \left(Y_i - \widehat{F}_0(d_{1i}, d_{2i}, d_{3i}) - \widehat{f}_{12}(d_{1i}, d_{2i}) - \widehat{f}_{13}(d_{1i}, d_{3i}) - \widehat{f}_{23}(d_{2i}, d_{3i})\right) \\ &\quad \times \mathbf{1}_{\{d_{1i}\neq 0 \ \& \ d_{2i}\neq 0 \ \& \ d_{3i}\neq 0\}} \end{aligned}$$

Similar to (2.18), the first term of (6.2) measures the goodness of fit, the third term, $J\left(f_{123}\right)$, measures the smoothness of the function $f_{123}\left(d_1, d_2, d_3\right)$, and the second term, λ, is a smoothing parameter that measures the trade off between the goodness of fit and the smoothness of the function f_{123}.

As in Kong and Lee's two drug case, the minimizer of $PRSS$ is necessarily a natural thin plate spline, although here it is a natural thin plate spline of order 3 [13]. As a thin plate spline of order 3, $f_{123}\left(d_1, d_2, d_3\right)$ can be expressed as a linear combination of the radial basis functions:

$$f_{123}\left(d_1, d_2, d_3\right) = \gamma_0 + \gamma_1 d_1 + \gamma_2 d_2 + \gamma_3 d_3 + \sum_{k=1}^{K} v_k \eta\left(\left\|\left(d_1, d_2, d_3\right)^T - \left(\kappa_{1k}, \kappa_{2k}, \kappa_{3k}\right)^T\right\|\right)$$

with the radial basis function:

$$\eta\left(r\right) = \frac{1}{8\pi} r^3$$

and with knots, $\left(\kappa_{1k}, \kappa_{2k}, \kappa_{3k}\right)^T$ $\left(k = 1, \ldots, K\right)$, which are all of the distinct values of the combination doses $\left(d_{1i}, d_{2i}, d_{3i}\right)^T$ $\left(i = 1, \ldots, n\right)$. The distance between any two combination doses is defined as the Euclidean distance, so the distance between a combination dose and a knot is:

$$\left\|\left(d_1, d_2, d_3\right)^T - \left(\kappa_{1k}, \kappa_{2k}, \kappa_{3k}\right)^T\right\| = \sqrt{\left(d_1 - \kappa_{1k}\right)^2 + \left(d_2 - \kappa_{2k}\right)^2 + \left(d_3 - \kappa_{3k}\right)^2}.$$

We can then define a $K \times K$ matrix $\Omega = \left[\left\|\left(\kappa_{1k}, \kappa_{2k}, \kappa_{3k}\right)^T - \left(\kappa_{1k'}, \kappa_{2k'}, \kappa_{3k'}\right)^T\right\|\right]_{1\leq k,k'\leq K}$,

a $K \times 4$ matrix $T^T = [1, \kappa_{1k}, \kappa_{2k}, \kappa_{3k}]_{1 \leq k \leq K}$, and a vector $v = (v_1, \ldots, v_k)^T$. The minimizer of (6.2) then satisfies $J(f) = v^T \Omega v$ and $Tv = 0$. We can then define:

$$
\begin{aligned}
Y_R &= [w_1, \ldots, w_n]^T, \\
X &= [1, d_{1i}, d_{2i}, d_{3i}]_{1 \leq i \leq n} \in R^{n \times 4}, \\
Z_1 &= \left[\eta \left(\| (d_{1i}, d_{2i}, d_{3i})^T - (\kappa_{1k}, \kappa_{2k}, \kappa_{3k})^T \| \right)_{1 \leq i \leq n} \right] \in R^{n \times K},
\end{aligned}
$$

and

$$\gamma = (\gamma_0, \gamma_1, \gamma_2, \gamma_3)^T.$$

Suppose FG is a QR decomposition of T^T such that F is a $K \times K$ orthogonal matrix, G is a $K \times 4$ upper triangular matrix, and $T^T = FG$. Let F_1 be the first four columns of F, and F_2 be the remaining $K - 4$ columns. Following the argument in Green and Silverman (1994, p. 166), we could then show that $Tv = 0$ if and only if v can be expressed as $F_2 \xi$, where ξ is a $K - 4$ vector. The minimizer of (6.2) is then essentially equivalent to minimizing

$$(Y_R - X\gamma - Z_1 F_2 \xi)^T (Y_R - X\gamma - Z_1 F_2 \xi) + \lambda \xi^T F_2^T \Omega F_2 \xi. \tag{6.3}$$

Similar to Kong and Lee's approach to minimizing (2.19) and estimating $f(d_1, d_2)$, we can minimize (6.3), select the smoothing parameter λ, and estimate $f_{123}(d_1, d_2, d_3)$ by using the technique of Ruppert, Wand and Carroll which uses a mixed effect model to estimate a thin plate spline [28]. Our approach closely follows that of Kong and Lee, and only differs in the dimensions of some of the vectors and matrices (e.g. here $\gamma_i \, (i = 0, 1, 2, 3)$ and $u_i \, (i = 1, \ldots, K - 4)$).

6.3.2 Generalized Cross Validation Smoothing

The second approach used to estimate $f_{123}(d_1, d_2, d_3)$ was to estimate it as a thin plate spline of order 3, using Generalized Cross Validation (GCV), which can be used to fit smoothing splines and thin plate splines of any order [7, 30]. The fitting was done using the R package rgcvpack [33], which uses the GCVPACK library [2] to do the actual fitting.

As part of the second approach, the original Kong and Lee method for estimating synergy between two drugs was modified to use GCV to fit the thin plate splines that estimate $f(d_1, d_2)$, instead of the Kong and Lee mixed model-based approach to fit the thin plate splines.

The Kong and Lee approach of estimating the standard error of $f(d_1, d_2)$ was also modified. The approach follows Kong and Lee in using a wild bootstrap, but was generalized to be expressed in terms of the smoothed, undersmoothed and oversmoothed estimates of $f(d_1, d_2)$, rather than the specific calculations Kong and Lee had used to estimate $f(d_1, d_2)$ and its undersmoothed and oversmoothed variants.

The approach can be summarized as follows:

Step 1. Fit the model based on the original observations, obtain $\widehat{F}_0(d_{1i}, d_{2i})$ and $\hat{f}(d_{1i}, d_{2i})$, along with $\hat{\lambda}$, where $\hat{\lambda}$, is the estimate of the smoothing parameter λ in the estimate of $\hat{f}$.

Step 2. Obtain the residuals from the undersmoothed estimation of $f(d_1, d_2)$, that is, $\hat{\epsilon}_i = Y_i - \hat{F}_0(d_{1i}, d_{2i}) - \hat{f}_{0.5\hat{\lambda}}(d_{1i}, d_{2i})$, where $\hat{f}_{0.5\hat{\lambda}}(d_{1i}, d_{2i})$ is the undersmoothed estimate of f, with smoothing parameter $0.5\hat{\lambda}$.

Step 3. Generate n i.i.d. (independent and identically distributed) random variables $\epsilon_1^*, \ldots, \epsilon_n^*$ with mean 0 and variance 1, for example, $\epsilon_i^* = -\frac{\sqrt{5}-1}{2}$ with probability $\frac{\sqrt{5}+1}{2\sqrt{5}}$ and $\epsilon_i^* = \frac{\sqrt{5}+1}{2}$ with probability $\frac{\sqrt{5}-1}{2\sqrt{5}}$.

Step 4. Obtain the fitted value from the oversmoothed estimation of $f(d_1, d_2)$, say, $Y_i^* = \hat{F}_0(d_{1i}, d_{2i}) + \hat{f}_{2\hat{\lambda}}(d_{1i}, d_{2i}) + \hat{\epsilon}_i\hat{\epsilon}_i^*$ for $i = 1, \ldots, n$, where $\hat{f}_{2\hat{\lambda}}(d_{1i}, d_{2i})$ is the oversmoothed estimate of f, with smoothing parameter $2\hat{\lambda}$.

Step 5. Fit the model using the generated data (d_{1i}, d_{2i}, Y_i^*) $(i = 1, \ldots, n)$, and then obtain the estimated function $f^*(d_1, d_2)$.

Step 6. Repeat step 2 to step 5 B (say, 50) times.

Denote the estimated $f(d_1, d_2)$ in the b^{th} $(b = 1, \ldots, B)$ iteration as $f^{*b}(d_1, d_2)$, and then estimate the standard deviation of $f(d_1, d_2)$ by:

$$\widehat{SD}^{*B}\left(\hat{f}(d_1, d_2)\right) = \left(\frac{1}{B}\sum_{b=1}^{B}\left(f^{*b}(d_1, d_2) - \hat{f}(d_1, d_2)\right)^2\right)^{\frac{1}{2}},$$

Then construct a $100\,(1-\alpha)\,\%$ confidence interval for $f\,(d_1, d_2)$:

$$\left[\hat{f}\,(d_1,d_2) - z_{\frac{\alpha}{2}} \times \widehat{SD}^{*B}\left(\hat{f}\,(d_1,d_2)\right), \hat{f}\,(d_1,d_2) + z_{\frac{\alpha}{2}} \times \widehat{SD}^{*B}\left(\hat{f}\,(d_1,d_2)\right)\right],$$

where $z_{\frac{\alpha}{2}}$ is the upper $\frac{\alpha}{2} \times 100\%$ percentile of the standard normal distribution, and $\hat{f}\,(d_1, d_2)$ is the smoothed estimate for $f\,(d_1, d_2)$.

6.4 Variance Estimation Method Description

Similar to Kong and Lee, the variance of $f_{12}\,(d_1, d_2)$, $f_{13}\,(d_1, d_3)$, $f_{23}\,(d_2, d_3)$, and $f_{123}\,(d_1, d_2, d_3)$ can be estimated using a wild bootstrap [8]. An overview of the procedure follows:

1. Fit the model using the original observations, obtaining $\widehat{F}_0(d_{1i}, d_{2i}, d_{3i})$, $\widehat{f_{12}}(d_{1i}, d_{2i})$, $\widehat{f_{13}}(d_{1i}, d_{3i})$, $\widehat{f_{23}}(d_{2i}, d_{3i})$, and $\widehat{f_{123}}(d_{1i}, d_{2i}, d_{3i})$, along with $\widehat{\lambda_{12}}$, $\widehat{\lambda_{13}}$, $\widehat{\lambda_{23}}$, and $\widehat{\lambda_{123}}$, where $\widehat{\lambda_k}$, $k \in \{123, 12, 13, 23\}$, is the estimate of the smoothing parameter λ in the estimate of $\widehat{f_k}$ (in (2.18) for f_{12}, f_{13} and f_{23}, in (6.2) for f_{123}).

2. Let

$$\begin{aligned}\widehat{\epsilon_i} &= Y_i - \widehat{F}_0(d_{1i}, d_{2i}, d_{3i}) \\ &\quad - \widehat{f}_{12,\,0.5\widehat{\lambda_{12}}}(d_{1i}, d_{2i}) - \widehat{f}_{13,\,0.5\widehat{\lambda_{13}}}(d_{1i}, d_{3i}) - \widehat{f}_{23,\,0.5\widehat{\lambda_{23}}}(d_{2i}, d_{3i}) \\ &\quad - \widehat{f}_{123,\,0.5\widehat{\lambda_{123}}}(d_{1i}, d_{2i}, d_{3i}),\end{aligned}$$

   where $\widehat{f}_{k,\,0.5\widehat{\lambda_k}}$, $k \in \{123, 12, 13, 23\}$, is the undersmoothed estimate of f_k, with smoothing parameter $0.5\widehat{\lambda_k}$.

3. Generate n i.i.d. random variables $\epsilon_1^*, ..., \epsilon_n^*$ with mean 0 and variance 1.

4. Obtain the "fitted" value Y_i^*

$$Y_i^* = \widehat{F}_0(d_{1i}, d_{2i}, d_{3i}) + \eta_i + \widehat{\epsilon}_i \epsilon_i^*$$

for $i = 1, ..., n$, where

$$\eta_i = \begin{cases} \widehat{f}_{12,\,2\widehat{\lambda_{12}}}(d_{1i}, d_{2i}) + \widehat{f}_{13,\,2\widehat{\lambda_{13}}}(d_{1i}, d_{3i}) & \\ \qquad + \widehat{f}_{23,\,2\widehat{\lambda_{23}}}(d_{2i}, d_{3i}) & \text{If } d_{1i} \neq 0 \text{ and } d_{2i} \neq 0 \text{ and } d_{3i} \neq 0, \\ \qquad + \widehat{f}_{123,\,2\widehat{\lambda_{123}}}(d_{1i}, d_{2i}, d_{3i}) & \\ \widehat{f}_{12,\,2\widehat{\lambda_{12}}}(d_{1i}, d_{2i}) & \text{If } d_{1i} \neq 0 \text{ and } d_{2i} \neq 0 \text{ and } d_{3i} = 0, \\ \widehat{f}_{13,\,2\widehat{\lambda_{13}}}(d_{1i}, d_{3i}) & \text{If } d_{1i} \neq 0 \text{ and } d_{2i} = 0 \text{ and } d_{3i} \neq 0, \\ \widehat{f}_{23,\,2\widehat{\lambda_{23}}}(d_{2i}, d_{3i}) & \text{If } d_{1i} = 0 \text{ and } d_{2i} \neq 0 \text{ and } d_{3i} \neq 0, \\ Y_i - \widehat{F}_0(d_{1i}, d_{2i}, d_{3i}) & \text{Otherwise.} \end{cases}$$

and where $\widehat{f}_{k,\,2\widehat{\lambda_k}}$, $k \in \{123, 12, 13, 23\}$, is the oversmoothed estimate of f_k, with smoothing parameter $2\widehat{\lambda_k}$.

A preliminary method for generating Y_i^* always used the first case of η_i, regardless of the values, of d_{1i}, d_{2i} and d_{3i}[26]. But that was found to have bias in some of the Y_i^* bootstrap samples. Further investigation showed that the same type of bias was present in the original Kong and Lee method, as described in Appendix C. To help reduce the bias for the extended three drug method, the two drug solution in Appendix C was extended for three drugs, resulting in the various cases of η_i being added, with the last case being especially important.

5. Fit the model using the generated data $(d_{1i}, d_{2i}, d_{3i}, Y_i^*)$ $(i = 1, ..., n)$, and then obtain the estimated functions $F_0^*(d_1, d_2, d_3)$, $f_{12}^*(d_1, d_2)$, $f_{13}^*(d_1, d_3)$, $f_{23}^*(d_2, d_3)$, and $f_{123}^*(d_1, d_2, d_3)$.

6. Repeat step 2 to step 5 B (say, 50) times.

Let $f_{123}^{*b}(d_1, d_2, d_3)$ be the estimate of the b^{th} iteration, the standard deviation for $f_{123}(d_1, d_2, d_3)$ can then be estimated by

$$\widehat{SD}^{*B}(\widehat{f}_{123}(d_1, d_2, d_3)) = \left(\frac{1}{B} \sum_{b=1}^{B} \left(f_{123}^{*b}(d_1, d_2, d_3) - \widehat{f}_{123}(d_1, d_2, d_3) \right)^2 \right)^{1/2},$$

and a $100(1-\alpha)\%$ confidence interval for $f_{123}(d_1, d_2, d_3)$ can be constructed as

$$\widehat{f}_{123}(d_1, d_2, d_3) \pm z_{\frac{\alpha}{2}} \times \widehat{SD}^{*B}(\widehat{f}_{123}(d_1, d_2, d_3)).$$

The standard deviations and confidence intervals for $f_{12}(d_1, d_2)$, $f_{13}(d_1, d_3)$, and $f_{23}(d_2, d_3)$ can be estimated in a similar fashion.

6.5 Response Surface Estimation

An R program was created to implement the extended Kong and Lee semiparametric method described in the previous section.

Five constructed data sets were analyzed using the program. Similar to the data sets constructed for the extended Plummer and Short method, the data sets corresponded to 5 different synergy scenarios: additivity between all 3 drugs, 2-way synergy between one pair of the drugs, 3-way synergy between all of the drugs, 2-way synergy between one pair of drugs combined with 3-way synergy between all of the drugs, and a combination of localized 3-way synergy and 3-way antagonism.

Each constructed data set consisted of generated measurements for combination treatments of 3 drugs. All 3 drugs had 7 dose levels. In addition to a dose of 0, the first drug had dose levels of 2.5, 5, 7.5, 10, 15, and 20. The second drug had levels one half of the first drug, while the third had levels one quarter of the first drug. The relative potency of the first drug to the second drug was defined as ρ_2:

$$\log(\rho_2) = \beta_2 + \beta_3 \log(D_2) \tag{6.4}$$

where D_2 is the solution to:

$$D_2 = d_2 + d_1/\rho_2$$

and $\beta_2 = \log 2$ and $\beta_3 = 0.02$. The relative potency of the first drug to the third drug was defined as ρ_3:

$$\log(\rho_3) = \beta_4 + \beta_5 \log(D_3) \tag{6.5}$$

where D_3 is the solution to:

$$D_3 = d_3 + d_1/\rho_3$$

and $\beta_4 = \log 3$ and $\beta_5 = 0.005$. All possible combinations of the 3 drugs at the given dose levels were used. For each combination of drugs where all 3 drugs were not zero, there were six generated results at each combination. For each combination of drugs

where one drug was zero, there were twelve generated results at each combination; the additional results were used to improve the estimates of the two-way drug synergy estimates.

The generated response variable was a proportion, such as the proportion of cells viable after the given treatment, a common end point in many nonclinical oncology studies. Because the analysis program assumes the response E is a proportion, it initially transforms it to be the transformed effect Y using the function $g(E) = \text{logit}\, E = \log \frac{E}{1-E}$. All generated responses were constructed on the transformed scale as y, and then transformed to be E on the original scale of responses using the function $g^{-1}(y) = \text{logit}^{-1} y = \frac{1}{1+\exp(-y)}$. A small amount of random noise, from a normal distribution with a mean of 0 and a standard deviation of 0.2, was added to each generated response value before its antilogit transformation.

Each constructed data set was constructed based on a five-component model specified in (6.1), with an additional random component:

$$y = F_0(d_1, d_2, d_3) + f_{12}(d_1, d_2) + f_{13}(d_1, d_3) + f_{23}(d_2, d_3) + f_{123}(d_1, d_2, d_3) + \epsilon \quad (6.6)$$

where:

$$\epsilon \sim N\left(0, 0.2^2\right)$$

and with the same conditions as (6.1):

$$\begin{aligned} f_{12}(d_1, 0) &= f_{12}(0, d_2) = f_{13}(d_1, 0) = f_{13}(0, d_3) = f_{23}(d_2, 0) = f_{23}(0, d_3) = 0, \\ f_{123}(d_1, d_2, 0) &= f_{123}(d_1, 0, d_3) = f_{123}(0, d_2, d_3) = 0. \end{aligned}$$

All five data sets were constructed based on (6.6), but with different functions used for f_{12}, f_{13}, f_{23} and f_{123}. A common F_0 function was used to construct all four data sets:

$$F_0(d_1, d_2, d_3) = \beta_0 + \beta_1 \log(d_1 + \rho_2 d_2 + \rho_3 d_3)$$

where $\beta_0 = 4$, $\beta_1 = -1$, and ρ_2 and ρ_3 were defined as in (6.4) and (6.5) respectively, with $\beta_2 = \log 2$, $\beta_3 = 0.02$, $\beta_4 = \log 3$, and $\beta_5 = 0.005$.

For each scenario, the constructed data set was analyzed by the extended Kong and Lee semiparametric program. Figures show the actual and fitted data for each

scenario, along with the residuals from the fitted model. Fitted results and residuals from intermediate steps are also shown. The figures of the estimated f_{12}, f_{13}, f_{23} and f_{123} functions include dashed lines showing where a 95% confidence interval for the fitted surface crosses the zero plane, indicating statistically significant synergy and/or antagonism; dashed blue lines are used to indicate synergy while dashed red lines are used to indicate antagonism.

6.5.1 First Scenario: Additivity

The constructed data set for the first scenario assumed all drugs were additive. The response was constructed using the following function definitions:

$$f_{12}(d_1, d_2) = 0$$

$$f_{13}(d_1, d_3) = 0$$

$$f_{23}(d_2, d_3) = 0$$

$$f_{123}(d_1, d_2, d_3) = 0$$

Figure 6.1 shows the results for the first constructed data set. Panels A through C of the figure show the fitted log dose-response curves for each individual drug, used alone. The log-dose response curves were used to construct initial estimates for the parametric part of the extended Kong and Lee semiparametric model.

Panels D through G in the figure show contour plots of the "observed" constructed data, with each panel showing the contour plot at a different dose of Drug A. Each plot shows the response contours for the given doses of Drug B and Drug C, on the X and Y axes respectively.

The contour lines in Panels D through G should generally be straight, reflecting the additivity between the drugs, but some of them are "jagged", especially at higher doses. There are likely two causes for this jaggedness. The first is that the high variability of the responses makes the lines more jagged; preliminary results that used much less variability when constructing the data produced much more straight lines[26]. The second may be with the contour plotting software itself. Some authors have proposed using GCVPACK to produce contour plots, in order "to produce smoothly varying

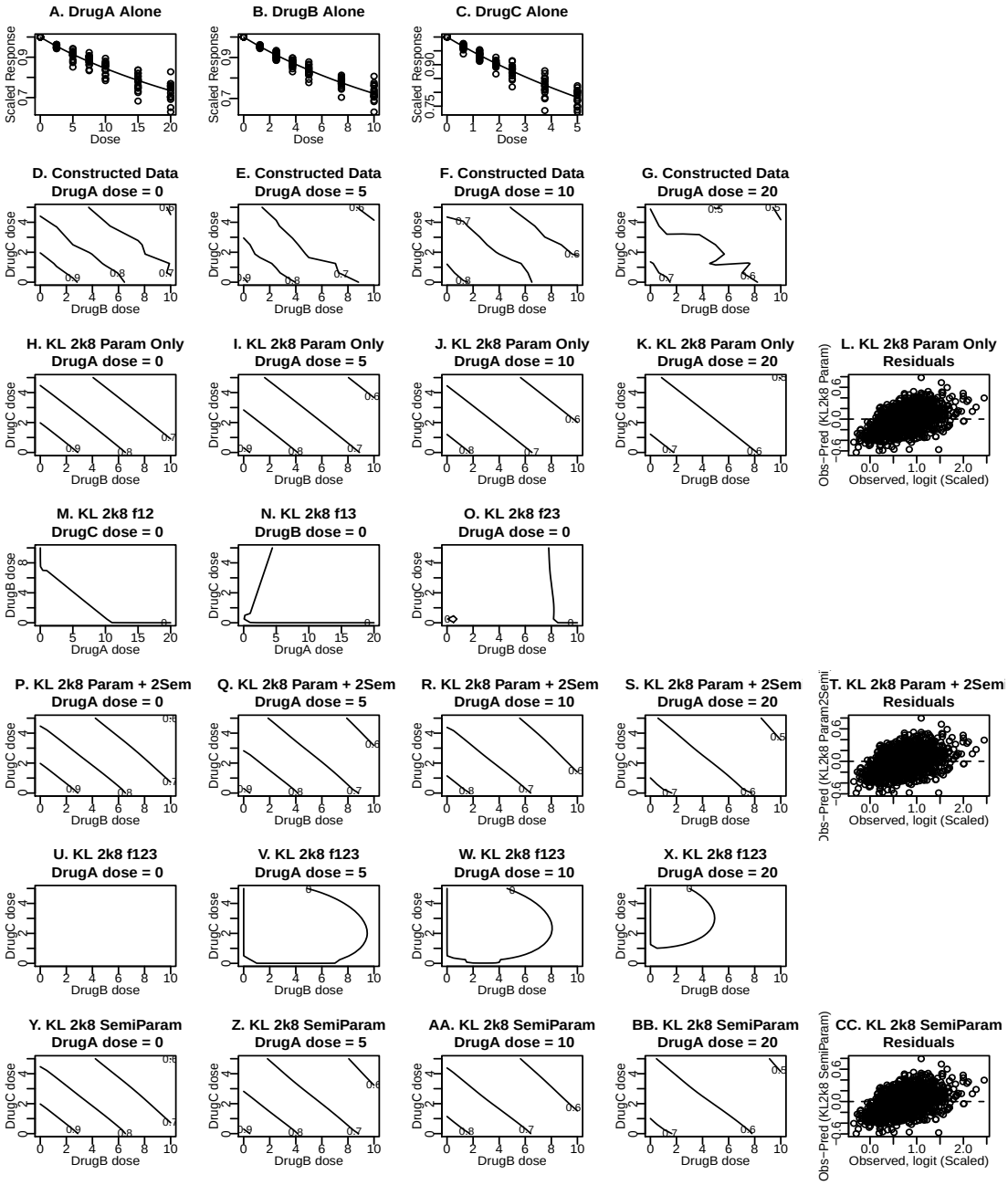

Figure 6.1: Results from first scenario, with all additive relations.

contour plots, with none of the jagged corners that plague many other interpolation methods [1]." This proposal was not tried here because GCVPACK is already being used to estimate the fitted response surface, and using the exact same method to fit the response surface and plot the "observed" surface, may cause the plots of the response surface to more closely resemble the plots of the "observed" surfaces.

The next five rows of panels reflect the results at two intermediate steps of the extended method, and the final results.

The first step of the extended method is to fit a parametric additive model to the data. The results from this step are shown in Panels H through L. Panels H through K show contour plots of the fitted model at different doses of Drug A. The contour lines in these panels are straight, reflecting the additive nature of the fitted parametric model. Because the "observed" data in this scenario actually is additive, the fitted contour lines in these panels closely correspond to most of the observed contour lines in Panels D through G, although some of the observed lines are more jagged, for reasons described above. Panel L shows the residuals from the fitted model, plotted against the observed (logit) response.

The second step of the extended Kong and Lee method is to make nonparametric estimates of f_{12}, f_{13}, and f_{23} in (6.1), which model the 2-way drug interactions. These estimates are then added to the parametric additive model fitted in the first step, to augment its fit.

Panels M through O show contour plots of f_{12}, f_{13}, and f_{23} respectively. Note that in Panels M and O the axes represent different drugs than in the other contour plots. These three plots show some random noise, but no statistically significant departure from the 0 plane.

Panels P through T show the results after augmenting the additive parametric model with the nonparametric estimates of the 2-way interactions, f_{12}, f_{13} and f_{23}. Panels P through S show contour plots of the augmented fitted model at different doses of Drug A. The contour lines in these panels are also generally straight, like those in Panels H through K. Because the "observed" data in this scenario actually is additive, the nonparametric estimates of 2-way synergy, f_{12}, f_{13}, and f_{23}, were essentially 0 and did

little to change the original parametric estimate shown in Panels H through K. Panel T shows the residuals from the augmented model, plotted against the observed (logit) response. The residuals show little change from those in Panel L, again reflecting the fact that the 2-way nonparametric estimates have done little to change the original additive parametric estimates.

The third and final step of the extended method is to make a nonparametric estimate of f_{123}, which models the 3-way drug interaction. This estimate is then added to the model from the second step to further augment its fit.

Panels U through X show contour plots of f_{123} at different levels of Drug A. Like the plots of f_{12}, f_{13} and f_{23}, these plots show no departure from the 0 contour.

Panels Y through CC show the results after augmenting the previous model with the nonparametric estimate of 3-way interaction, f_{123}. Panels Y through BB show contour plots of the further augmented fitted model at different doses of Drug A. The contour lines in these panels are also generally straight, because the "observed" data in this scenario actually is additive. As in the previous step, the nonparametric estimates f_{123} of 3-way synergy are essentially 0 and do little to change the estimates from the previous step. Panel CC shows the residuals from the further augmented model, plotted against the observed (logit) response. The residuals also show little change from those in Panel T, again reflecting the fact that the 3-way nonparametric estimates have done little to change the estimates from the previous step.

6.5.2 Second Scenario: Two-Way Synergy

The constructed data set for the second scenario assumed that there was 2-way synergy between drugs 2 and 3, but all other relations were additive. The response was constructed using these function definitions:

$$
\begin{aligned}
f_{12}(d_1, d_2) &= 0 \\
f_{13}(d_1, d_3) &= 0 \\
f_{23}(d_2, d_3) &= -0.2\sqrt{\log(d_2 + 1)\log(d_3 + 1)} \\
f_{123}(d_1, d_2, d_3) &= 0
\end{aligned}
$$

Figure 6.2 shows the results for the second constructed data set. As in the previous scenario, the log-dose response curves in Panels A through C were used to construct initial estimates for the parametric part of the extended Kong and Lee model.

In this scenario, the contour plots of the "observed" data in Panels D through G are now curved, reflecting the 2-way synergy between Drug B and Drug C. As in the previous scenario, the next three rows show the fitted model after the first two initial steps of the extended Kong and Lee model, and after the final step.

Panels H through L show the results after the first step, of fitting a parametric additive model. Panels H through K show contour plots of the additive model at different doses of Drug A. As in the previous scenario, the lines here are straight. But in this scenario, they do not match the observed data very well, which is reflected in the larger residuals in panel L.

Panels M through O show contour plots of f_{12}, f_{13}, and f_{23} respectively. Although Panels M and N still show no departure from the 0 plane for f_{12} and f_{13} respectively, the plot of f_{23} in Panel O reflects the non-zero definition of f_{23} in this scenario. The dashed blue line shows the contour where the upper limit of the 95% confidence interval of the f_{23} estimate crosses the zero plane, indicating statistically significant synergy for all dose combinations whose contours are at or below that contour.

Panels P through T show the results after augmenting the additive parametric model with the nonparametric estimates of the 2-way interactions, f_{12}, f_{13} and f_{23}. Panels P through S show contour plots at different doses of Drug A. Now the contour lines are curved, reflecting the 2-way synergy between Drug B and Drug C, which has been detected. The plots in Panels P through S are closer in shape to the observed data in Panels D through G than were the plots in Panels H through K. The residuals in Panel T are smaller than those in Panel L, reflecting the improvement in the model that has been made by the nonparametric estimates of the 2-way drug interactions.

Panels U through X show contour plots of f_{123} at different levels of Drug A. As in the previous scenario, these plots are flat, and near the 0 plane.

Panels Y through CC show the results after augmenting the previous model with the nonparametric estimate of 3-way interaction, f_{123}. Panels Y through BB show contour

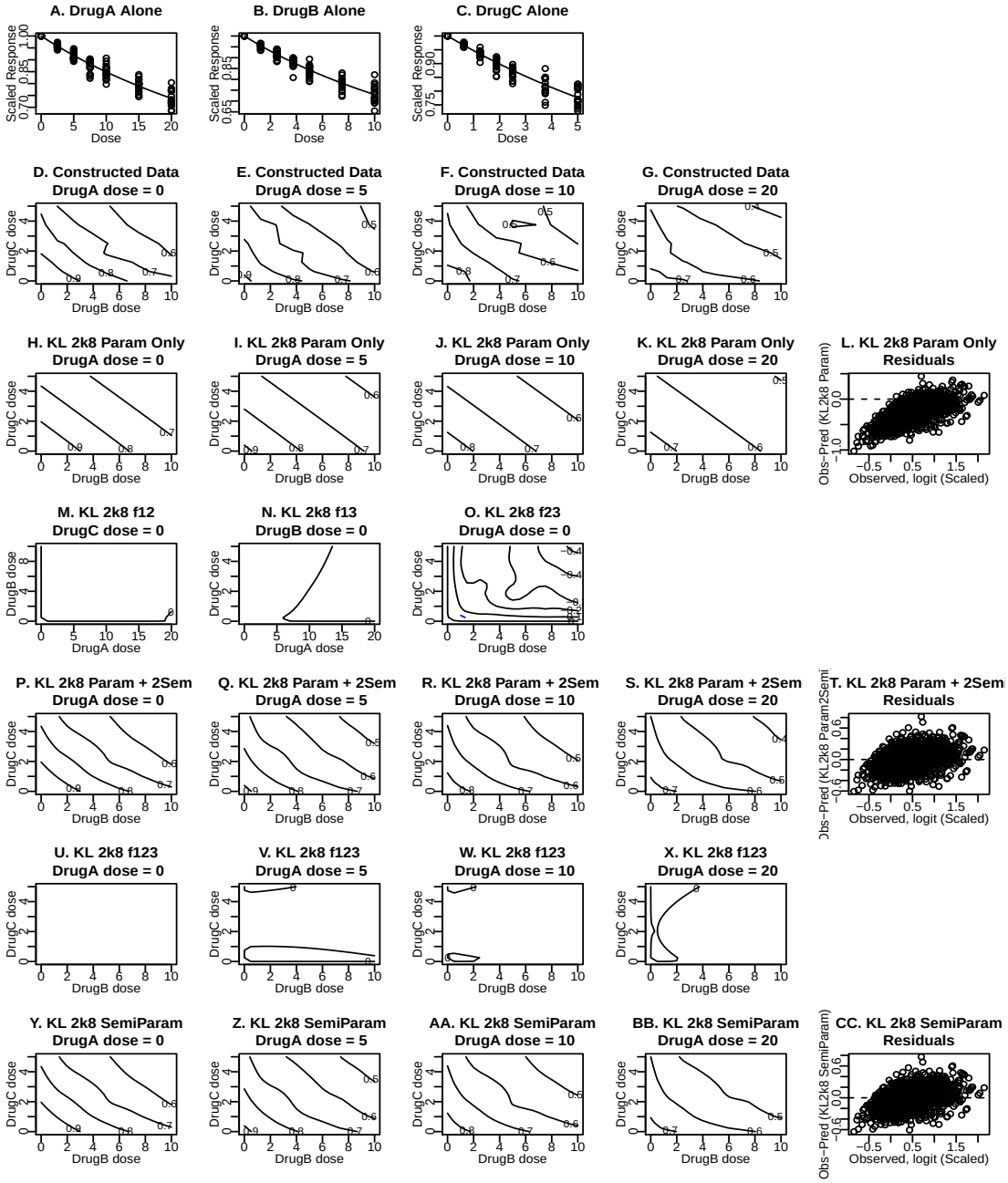

Figure 6.2: Results from second scenario, with 2-way synergy between Drug B and Drug C, but additivity for all other relations.

plots of the further augmented fitted model at different doses of Drug A. The contour lines in these panels are little changed from those of the previous step, shown in Panels P through S, showing that the estimation of f_{123} is essentially nothing and changes the model very little. The residuals in Panel CC also show little improvement from those of the previous step, in Panel T.

6.5.3 Third Scenario: Three-Way Synergy

The constructed data set for the third scenario assumed that there was 3-way synergy between all 3 drugs, but all 2-way relations were additive. The response was constructed using these function definitions:

$$f_{12}(d_1, d_2) = 0$$

$$f_{13}(d_1, d_3) = 0$$

$$f_{23}(d_2, d_3) = 0$$

$$f_{123}(d_1, d_2, d_3) = -0.1\left(\log(d_1 + 1)\log(d_2 + 1)\log(d_3 + 1)\right)^{\frac{1}{3}}$$

Figure 6.3 shows the results for the third constructed data set. As in the previous scenarios, the log-dose response curves in Panels A through C were used to construct initial estimates for the parametric part of the extended Kong and Lee model.

Also as in the previous scenario, the contour plots of the "observed" data in Panels E through G are still curved, reflecting the 3-way synergy between Drug A, Drug B and Drug C. But here the lines in Panel D are straight, reflecting the fact that this is 3-way synergy that depends on Drug A being present. In the previous scenario, the lines in Panel D were curved, because the 2-way synergy between Drug B and Drug C was present even in the absence of Drug A. As in the previous scenario, the next five rows show the fitted model after the first two initial steps of the extended Kong and Lee model, and after the final step.

Panels H through L show the results after the first step, of fitting a parametric additive model. Panels H through K show contour plots of the additive model at different doses of Drug A, and as in the previous scenarios, the lines here are straight. But in this scenario, they do not match the observed data very well, except for Panel

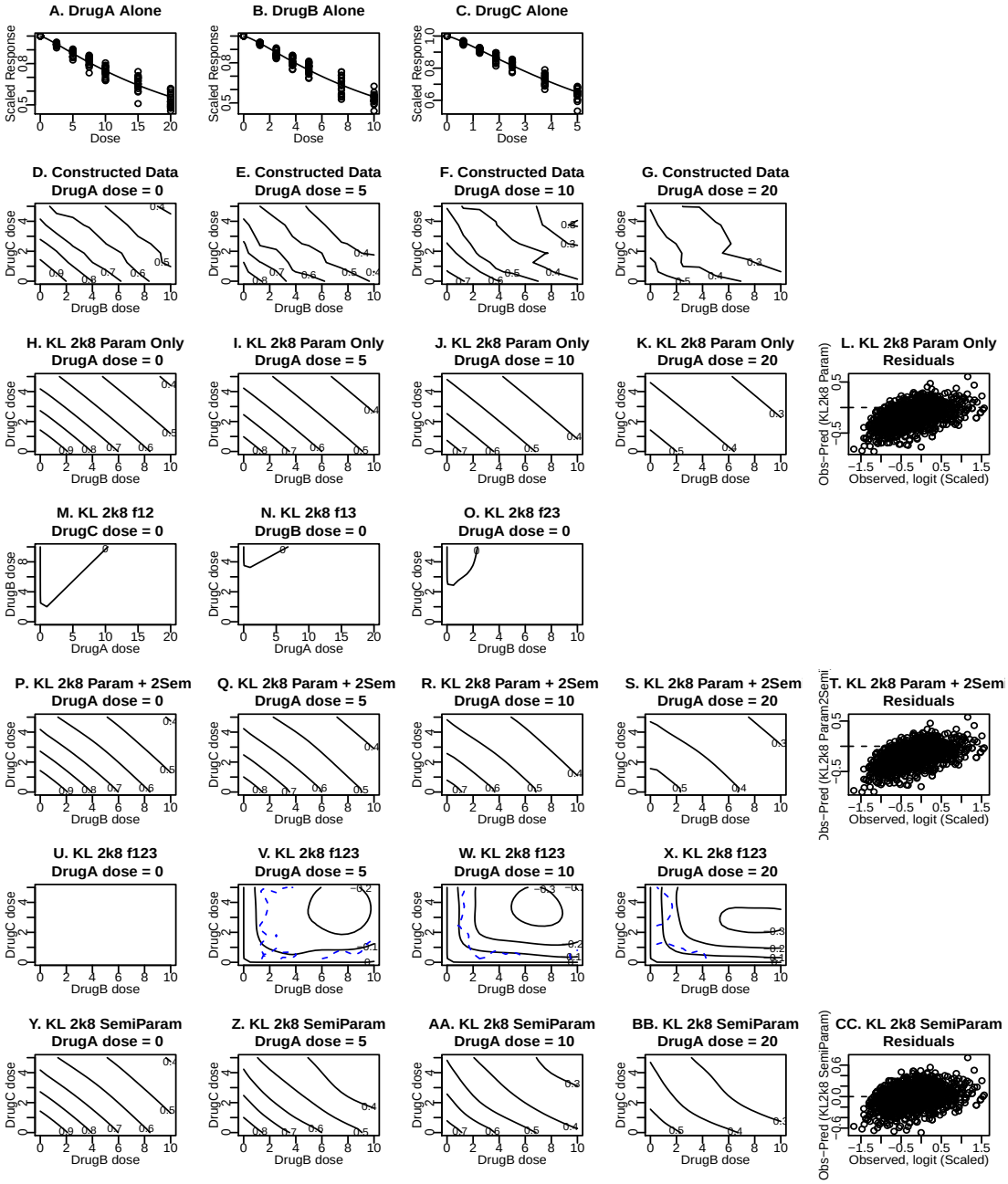

Figure 6.3: Results from third scenario, with 3-way synergy between Drug A, Drug B and Drug C, but additivity between each pair of drugs.

H where Drug A is absent and there is no interaction. This poor fit is reflected in the large residuals in panel L.

Panels M through O show contour plots of f_{12}, f_{13}, and f_{23} respectively. As in the first scenario, all three panels show no departure from the 0 plane.

Panels P through T show the results after augmenting the additive parametric model with the nonparametric estimates of the 2-way interactions, f_{12}, f_{13} and f_{23}. Panels P through S show contour plots at different doses of Drug A. Here the lines are still straight, reflecting the fact that the estimates of f_{12}, f_{13} and f_{23} did not detect any 2-way synergy between any of the drugs. The residuals in Panel T are essentially unchanged from those in Panel L, also reflecting the fact that augmenting the model with 2-way interactions has done little or nothing to improve it.

Panels U through X show contour plots of f_{123} at different levels of Drug A. Unlike the previous scenarios, these plots are not flat, reflecting the non-zero definition of f_{123} in this scenario. The dashed blue lines show the contours where the upper limit of the 95% confidence interval of the f_{123} estimate crosses the zero plane, indicating statistically significant synergy for all dose combinations whose contours are at or below that contour.

Panels Y through CC show the results after augmenting the previous model with the nonparametric estimate of 3-way interaction, f_{123}. Panels Y through BB show contour plots of the further augmented fitted model at different doses of Drug A. Here the lines in the contour plots in Panels Z through BB are now curved, accurately reflecting the 3-way synergy that has been detected, and matching the original data in Panels G through I. The lines on contour plot in Panel Y are still straight, reflecting the fact that the synergy is only present when all 3 drugs are present, and matching the original data in Panel D. Although the residuals in Panel CC do not look much smaller than those in the previous steps, their mean square has actually been reduced from 0.055 in Panel T, to 0.040 in Panel CC, indicating how much the model has been improved by the final step.

6.5.4 Fourth Scenario: Two-Way Synergy and Three-Way Synergy

The constructed data set for the fourth scenario assumed that there was 2-way synergy between one pair of drugs and 3-way synergy between all 3 drugs. The response was constructed using these function definitions:

$$
\begin{aligned}
f_{12}(d_1, d_2) &= 0 \\
f_{13}(d_1, d_3) &= 0 \\
f_{23}(d_2, d_3) &= -0.2\sqrt{\log(d_2+1)\log(d_3+1)} \\
f_{123}(d_1, d_2, d_3) &= -0.1\left(\log(d_1+1)\log(d_2+1)\log(d_3+1)\right)^{\frac{1}{3}}
\end{aligned}
$$

Figure 6.4 shows the results for the fourth constructed data set. As in the previous scenarios, the log-dose response curves in Panels A through C were used to construct initial estimates for the parametric part of the extended Kong and Lee model.

As in both previous scenarios, the contour plots of the "observed" data in Panels E through G are still curved, reflecting the 2-way synergy between Drug B and Drug C, and the 3-way synergy between Drug A, Drug B and Drug C. Unlike the previous scenario (but like the 2nd scenario) the lines in Panel D are curved, reflecting the fact that there is 2-way synergy that is present even when Drug A is absent. In the previous scenario, the lines in Panel D were straight, because the 3-way synergy between Drug A, Drug B and Drug C depended on the presence of Drug A.

As in the previous scenarios, the next five rows show the fitted model after the first two initial steps of the extended Kong and Lee model, and after the final step.

Panels H through L show the results after the first step, of fitting a parametric additive model. Panels H through K show contour plots of the additive model at different doses of Drug A, and as in the previous scenarios, the lines here are straight. In this scenario, they do not match the observed data very well and this poor fit is reflected in the large residuals in panel L.

Panels M through O show contour plots of f_{12}, f_{13}, and f_{23} respectively. Although Panels M and N still show no departure from the 0 lane for f_{12} and f_{13} respectively, the plot of f_{23} in Panel O reflects the non-zero definition of f_{23} in this scenario. The

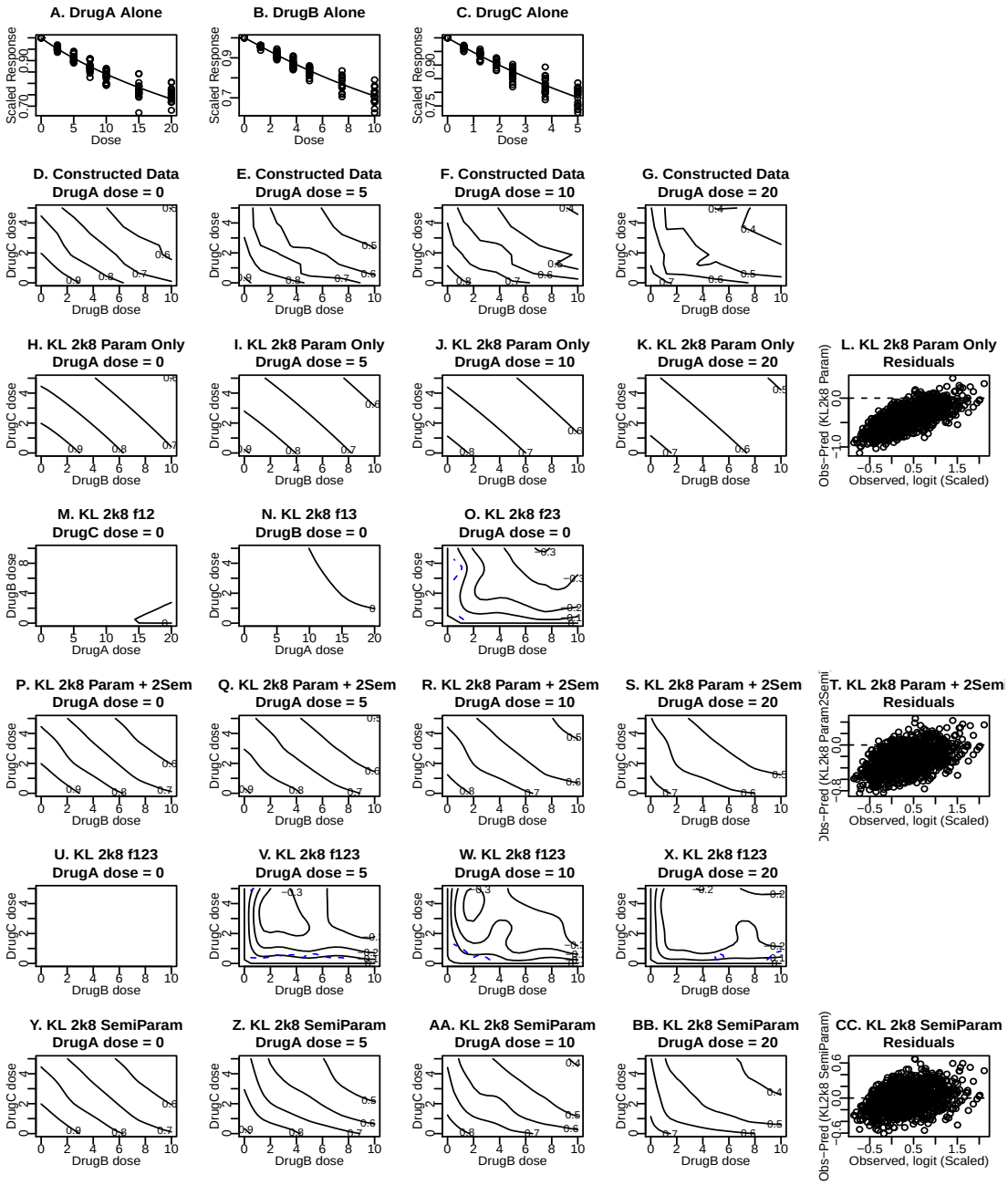

Figure 6.4: Results from fourth scenario, with 2-way synergy between Drug B and Drug C, and 3-way synergy between Drug A, Drug B and Drug C.

dashed blue line shows the contour where the upper limit of the 95% confidence interval of the f_{23} estimate crosses the zero plane, indicating statistically significant synergy for all dose combinations whose contours are at or below that contour.

Panels P through T show the results after augmenting the additive parametric model with the nonparametric estimates of the 2-way interactions, f_{12}, f_{13} and f_{23}. Panels P through S show contour plots at different doses of Drug A. Here the lines are curved, reflecting the 2-way synergy between Drug B and Drug C, which has been detected. The plots in Panels P through S are closer in shape to the observed data in Panels D through G than were the plots in Panels H through K, although they still do not quite match. The residuals in Panel T are smaller than those in Panel L, reflecting the improvement in the model that has been made by the nonparametric estimates of the 2-way drug interactions, but they still exhibit a pattern and do not show a very good fit.

Panels U through X show contour plots of f_{123} at different levels of Drug A. As in the previous scenario, these plots are not flat, reflecting the non-zero definition of f_{123} in this scenario. The dashed blue lines show the contours where the upper limit of the 95% confidence interval of the f_{123} estimate crosses the zero plane, indicating statistically significant synergy for all dose combinations whose contours are at or below that contour.

Panels Y through CC show the results after augmenting the previous model with the nonparametric estimate of 3-way interaction, f_{123}. Panels Y through BB show contour plots of the further augmented fitted model at different doses of Drug A. Here the lines in the contour plots in Panels Y through BB are now curved, accurately reflecting the 2-way and 3-way synergy that has been detected, and matching the original data in Panels G through I. Although the residuals in Panel CC do not look much smaller than those in the previous steps, their mean square has actually been reduced from 0.066 in Panel T, to 0.041 in Panel CC, indicating how much the model has been improved by the final step.

6.5.5 Fifth Scenario: Local Three-Way Synergy and Antagonism

The constructed data set for the fifth scenario assumed that there was local 3-way synergy between all 3 drugs in one region, where the dose of the first drug was less than 15, but local 3-way antagonism between all 3 drugs in another region, where the dose of the first drug was greater than 15; all 2-way relations were assumed to be additive. The response was constructed using these function definitions:

$$\begin{aligned} f_{12}(d_1, d_2) &= 0 \\ f_{13}(d_1, d_3) &= 0 \\ f_{23}(d_2, d_3) &= 0 \\ f_{123}(d_1, d_2, d_3) &= 0.2h(d_1)\left(\log(d_1+1)\log(d_2+1)\log(d_3+1)\right)^{\frac{1}{3}} \end{aligned}$$

where:

$$h(x) = \min\left(\max(x-15, -0.5), 0.4\right)$$

Figure 6.5 shows the results for the fifth constructed data set. As in the previous scenarios, the log-dose response curves in Panels A through C were used to construct initial estimates for the parametric part of the extended Kong and Lee model.

In this scenario, the contour plot of the "observed" data in Panel D are roughly straight, reflecting the fact that this synergy is 3-way synergy and which is not present when Drug A is absent. The contour plots in Panels E and F, the region of local 3-way synergy, are curved "down" and are similar to those in the previous scenario, which had global 3-way synergy. But the contour plot in Panel G, in the region of local 3-way antagonism, is curved "up" (with a fair amount of "jaggedness"), in contrast to the plot in Panel G of the previous scenario. As in the previous scenarios, the next five rows show the fitted model after the first two initial steps of the extended Kong and Lee model, and after the final step.

Panels H through L show the results after the first step, of fitting a parametric additive model. Panels H through K show contour plots of the additive model at different doses of Drug A, and as in the previous scenarios, the lines here are straight. And as in the previous two scenarios, they do not match the observed data very well,

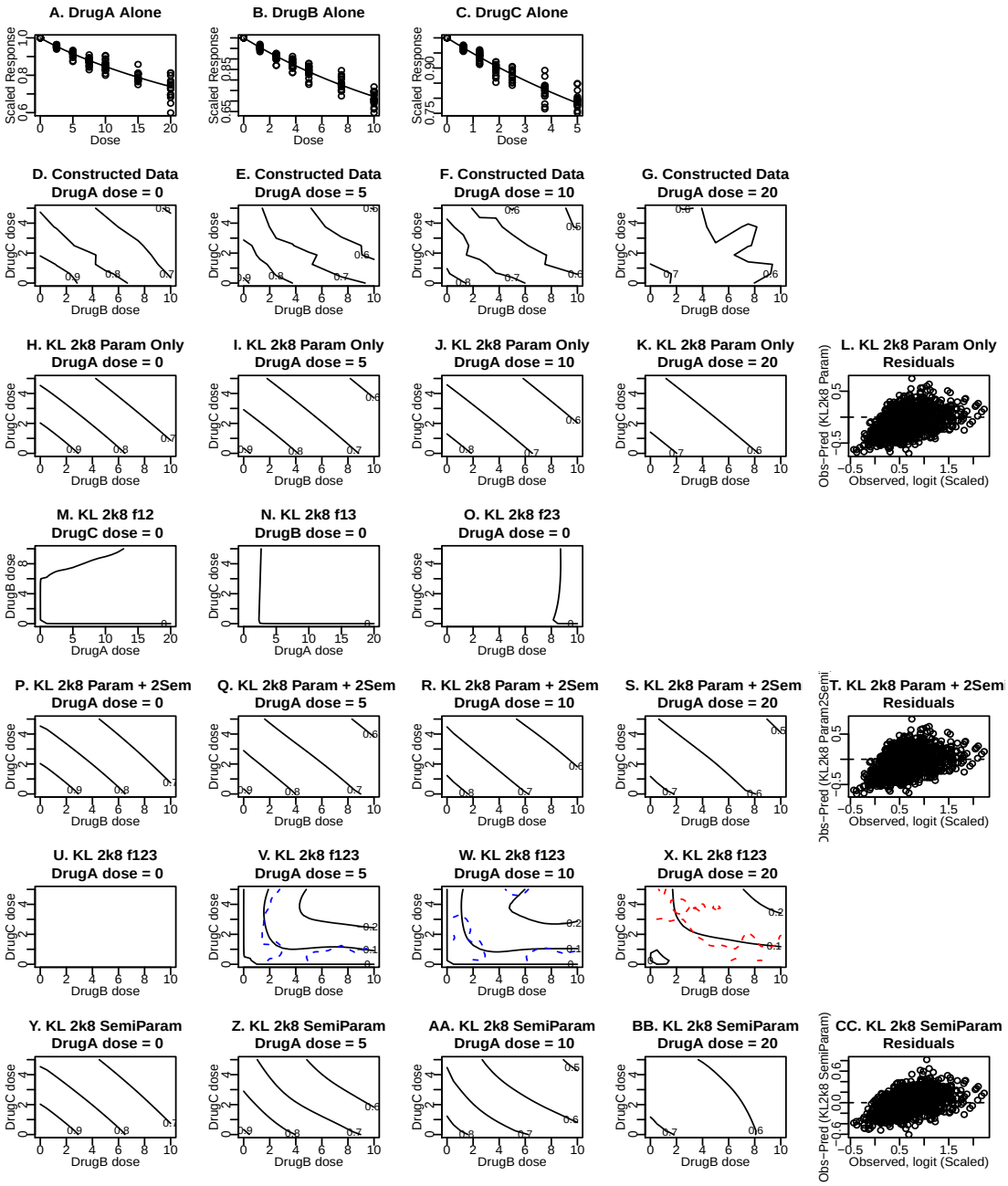

Figure 6.5: Results from fifth scenario, with local 3-way synergy in one region and local 3-way antagonism in another.

except for Panel H where Drug A is absent and there is no interaction. This poor fit is reflected in the large residuals in panel L.

Panels M through O show contour plots of f_{12}, f_{13}, and f_{23} respectively. As in the first and third scenarios, all three panels show no departure from the 0 plane.

Panels P through T show the results after augmenting the additive parametric model with the nonparametric estimates of the 2-way interactions, f_{12}, f_{13} and f_{23}. Panels P through S show contour plots at different doses of Drug A. Here the lines are still straight, reflecting the fact that the estimates of f_{12}, f_{13} and f_{23} did not detect any 2-way synergy between any of the drugs. The residuals in Panel T are essentially unchanged from those in Panel L, also reflecting the fact that augmenting the model with 2-way interactions has done little or nothing to improve it.

Panels U through X show contour plots of f_{123} at different levels of Drug A. As in the previous scenario, these plots are not flat, reflecting the non-zero definition of f_{123} in this scenario. As in the previous scenario, the contour lines in Panels V and W are negative, and the dashed blue lines show the contours where the upper limit of the 95% confidence interval of the f_{123} estimate crosses the zero plane, indicating statistically significant synergy for all dose combinations whose contours are at or below that contour. But in Panel X, the region of antagonism, the contour lines are at positive values, and the dashed red lines show the contours where the lower limit of the 95% confidence interval of the f_{123} estimate crosses the zero plane, indicating statistically significant antagonism for all dose combinations whose contours are at or above that contour.

Panels Y through CC show the results after augmenting the previous model with the nonparametric estimate of 3-way interaction, f_{123}. Panels Y through BB show contour plots of the further augmented fitted model at different doses of Drug A. Here the lines in the contour plots in Panels Z through BB are now curved, and they are curved in the proper "direction" accurately reflecting the 3-way synergy and 3-way antagonism that has been detected, and closely matching the original data in Panels E through G. The lines in contour plot in Panel Y are still straight, reflecting the fact that the synergy or antagonism is only present when all 3 drugs are present, and closely matching the

original data in Panel D. Although the residuals in Panel CC are not necessarily visibly smaller than those in the previous steps, their mean square has been improved from 0.048 to 0.041, showing how the model has been improved by this final step.

The handling of this scenario by the extended Kong and Lee method shows how this method is able to detect local synergy and antagonism, unlike the extended Plummer and Short method, which could only detect global synergy or antagonism.

6.6 Simulations to Evaluate Goodness of Fit and Confidence Intervals

Simulations were used to evaluate the goodness of fit of the extended Kong and Lee model and its confidence intervals, using a process similar to that used in Kong and Lee [20].

The simulated data was constructed similarly to that used in Section 6.5 for Response Surface Estimation, using the same five-component model:

$$y = F_0(d_1, d_2, d_3) + f_{12}(d_1, d_2) + f_{13}(d_1, d_3) + f_{23}(d_2, d_3) + f_{123}(d_1, d_2, d_3) + \epsilon$$

where:

$$\epsilon \sim N\left(0, \sigma_i^2\right)$$

and with the same conditions as (6.1):

$$\begin{aligned} f_{12}(d_1, 0) &= f_{12}(0, d_2) = f_{13}(d_1, 0) = f_{13}(0, d_3) = f_{23}(d_2, 0) = f_{23}(0, d_3) = 0, \\ f_{123}(d_1, d_2, 0) &= f_{123}(d_1, 0, d_3) = f_{123}(0, d_2, d_3) = 0. \end{aligned}$$

The same F_0 function was used to construct the simulated data sets:

$$F_0(d_1, d_2, d_3) = \beta_0 + \beta_1 \log(d_1 + \rho_2 d_2 + \rho_3 d_3)$$

where $\beta_0 = 4$, $\beta_1 = -1$, and ρ_2 and ρ_3 were defined as in (6.4) and (6.5) respectively, with $\beta_2 = \log 2$, $\beta_3 = 0.02$, $\beta_4 = \log 3$, and $\beta_5 = 0.005$.

Simulated data was constructed using the marginal single drug dose-response curves of the data set from the first scenario of Section 6.5, known "true" functions f_{123}, f_{12}, f_{13}, and f_{23} for the nonparametric parts of the model, and additional random

components. Seven different random components were used, depending on whether the combination of drugs included all 3 drugs, or only a subset of them; the random components will be described in greater detail below. The simulated data used the same dose levels as in the original constructed data sets.

The "true" functions of the semiparametric parts of the model were defined as below:

$$\begin{aligned} f_{12}(d_1, d_2) &= 0 \\ f_{13}(d_1, d_3) &= 0 \\ f_{23}(d_2, d_3) &= -0.2\sqrt{\log(d_2+1)\log(d_3+1)} \\ f_{123}(d_1, d_2, d_3) &= 0.4h(d_1)\left(\log(d_1+1)\log(d_2+1)\log(d_3+1)\right)^{\frac{1}{3}} \end{aligned}$$

where:

$$h(x) = \min\left(\max(x-15, -0.5), 0.4\right)$$

The true functions model local 3-way synergy and local 3-way antagonism, as well as global 2-way synergy between one pair of drugs. There is local 3-way synergy between all 3 drugs in the region where the dose of the first drug is less than 15, but local 3-way antagonism between all 3 drugs in the region where the dose of the first drug is greater than 15. There is global 2-way synergy between drug 2 and drug 3; the other 2-way relations are additive. The functions model a combination of the fourth scenario (2-way and 3-way global synergy) and fifth scenario (3-way local synergy and local antagonism) in the previous section.

A "true" logit-scale response for each dose combination was constructed as the sum of an additive model and the appropriate f_{123}, f_{12}, f_{13} and f_{23} functions. The additive model was constructed in the same manner as it was for the first scenario in Section 6.5. For dose combinations where only one drug's dose was non-zero, the true response was calculated using only the additive model. For dose combinations where two drugs' doses were non-zero, the response was calculated as the sum of the additive model and the appropriate f_{12}, f_{13} or f_{23} function. For dose combinations where all three drugs' doses were non-zero, the true response was calculated as the sum of the additive model and the f_{12}, f_{13}, f_{23} and f_{123} functions.

A random component was then added to the "true" logit-scale responses. The random component was taken from a normal distribution with a mean of 0 and a standard deviation that depended on which doses for that response were non-zero. Seven different standard deviations were used, σ_1, σ_2, ..., σ_7, one each for the three cases where only one drug was non-zero, one each for the three cases where only two of the drugs were non-zero, and one for the case where all three drugs were non-zero. For the three standard deviations in the cases where only one drug was non-zero, σ_1, σ_2, and σ_3, the standard deviation was the MSE from a linear regression model of the logit response on log dose for the Section 6.5 first scenario data of that drug alone ($\sigma_1 = 0.2342$, $\sigma_2 = 0.1694$, $\sigma_3 = 0.2026$). For the standard deviations of the cases where two drugs were non-zero, σ_4, σ_5, and σ_6, the standard deviation was the estimated MSE of the nonparametric function of those two doses (either f_{12}, f_{13} or f_{23}), estimated using the Section 6.5 fourth scenario data ($\sigma_4 = 0.1977$, $\sigma_5 = 0.1998$, $\sigma_6 = 0.2015$). For the standard deviation of the case where all three drugs were non-zero, σ_7, the standard deviation was the estimated MSE of the nonparametric function f_{123}, again estimated using the Section 6.5 fourth scenario data ($\sigma_7 = 0.2014$).

To test the goodness of fit and confidence intervals, multiple plates at a time were simulated, with the same true logit-scale responses, but with different random components. For dose combinations where all three drugs were present, three plates were simulated, for three repetitions of that dose combination (each with a different random component). Because the dose combinations with only one or two drugs present are critical for accurately estimating the parametric additive model and the 2-way nonparametric functions, six plates (or repetitions) were simulated for each dose combination where only one or two drugs were present. One hundred sets of simulated plates were generated.

Each set of simulated plates was fitted using the extended 3-drug Kong and Lee semiparametric method and their f_{12}, f_{13}, f_{23} and f_{123} functions were estimated. Confidence intervals for the functions were estimated using the wild bootstrap-based method, with 49 bootstrap samples for each set of simulated plates.

Figures 6.6 and 6.7 show the results of the fit of the simulations, while Figures 6.8

and 6.9 show the results of the confidence intervals for one of the simulation runs.

Figure 6.6 shows the results of estimating f_{12}, f_{13} and f_{23}. Each of these functions is a function of two variables, the dose levels of two of the drugs, and corresponds to a surface in 3-dimensional space. Showing multiple surfaces in a single graph of 3-dimensional space would be difficult to read, so multiple 2-dimensional cross-sections of the surface are shown at different dose levels.

Panels A through E of Figure 6.6 show the true and estimated curves of f_{12}. f_{12} is a function of d_1 and d_2. Each panel shows a cross section of the surface at a different level of d_1, with d_2 plotted on the X-axis, and the value of f_{12} at the corresponding values of d_1 and d_2 plotted on the Y-axis. In each panel, the true curve of f_{12} is shown as a solid black line and the estimated curves of f_{12} from the simulations are shown as dashed color lines. In most cases, the estimated curves are fairly close to the true curves.

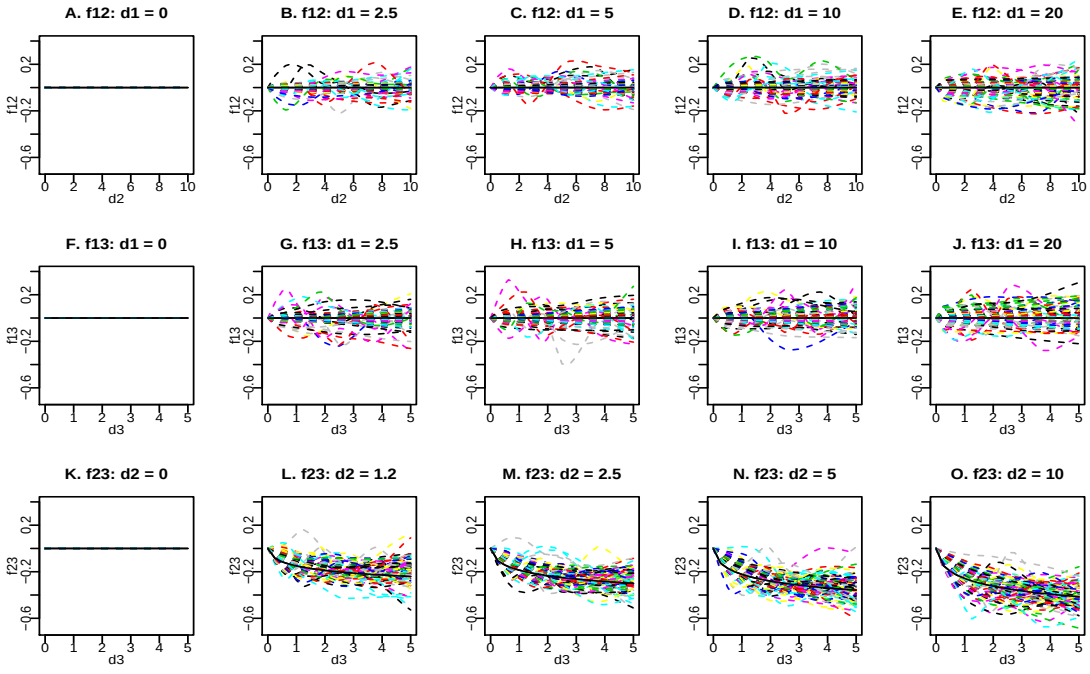

Figure 6.6: Estimated values of f_{12}, f_{13} and f_{23} from the simulations.

Panels F through J of Figure 6.6 show the true and estimated curves of f_{13}. f_{13} is a function of d_1 and d_3. Each panel shows a cross section of the surface at a different

level of d_1, with d_3 plotted on the X-axis, and the value of f_{13} at the corresponding values of d_1 and d_3 plotted on the Y-axis. In each panel, the true curve of f_{13} is shown as a solid black line and the estimated curves of f_{13} from the simulations are shown as dashed color lines. In most cases, the estimated curves are fairly close to the true curves.

Panels K through O of Figure 6.6 show the true and estimated curves of f_{23}. f_{23} is a function of d_2 and d_3. Each panel shows a cross section of the surface at a different level of d_2, with d_3 plotted on the X-axis, and the value of f_{23} at the corresponding values of d_2 and d_3 plotted on the Y-axis. In each panel, the true curve of f_{23} is shown as a solid black line and the estimated curves of f_{23} from the simulations are shown as dashed color lines. In most cases, the estimated curves are fairly close to the true curves.

Figure 6.7 shows the results of estimating f_{123}. f_{123} is a function of three variables, the dose levels of the three drugs, and corresponds to a surface in 4-dimensional space. It is shown here as multiple two-dimensional cross sections of the surface, with each panel showing the cross section at different levels of d_1 and d_2. Each panel then shows the cross section with d_3 plotted on the X-axis and the value of f_{123} at the corresponding values of d_1, d_2, and d_3 shown on the Y-axis. In each panel, the true curve of f_{123} is shown as a solid black line and the estimated curves of f_{123} from the simulations are shown as dashed color lines. In all cases, the estimated curves are fairly close to the true curves.

Figure 6.8 shows the estimated confidence intervals of f_{12}, f_{13} and f_{23}, for one of the simulation runs. Each of these functions is a function of two variables, the dose levels of two of the drugs, and corresponds to a surface in 3-dimensional space. Showing a surface and its confidence interval in a single graph of 3-dimensional space would be difficult to read, so multiple 2-dimensional cross-sections of the surface are shown at different dose levels, in the same manner as the estimated fits were shown in Figure 6.6.

Panels A through E of Figure 6.8 show the true and estimated curves of f_{12} for one simulation run (the same run shown in Panels A through J), along with the estimated confidence interval of f_{12}. f_{12} is a function of d_1 and d_2. Each panel shows a cross

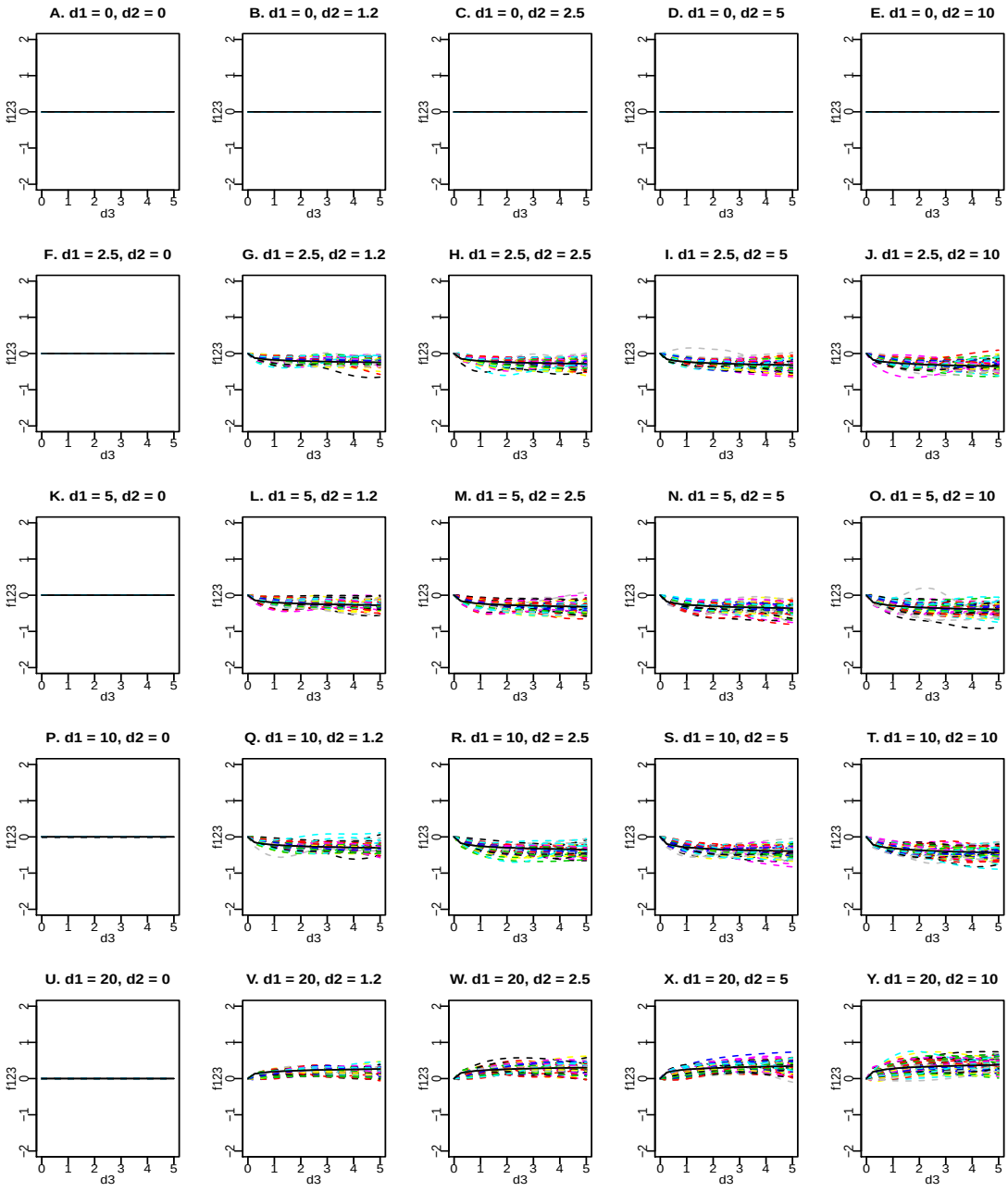

Figure 6.7: Estimated values of f_{123} from the simulations.

section of the surface at a different level of d_1, with d_2 plotted on the X-axis, and the value of f_{12} at the corresponding values of d_1 and d_2 plotted on the Y-axis. In each panel, the true curve of f_{12} is shown as a solid black line, the estimated curve of f_{12} from the simulation run is shown as a dashed black line, and the estimated confidence interval of f_{12} from the simulation run is shown with dotted black lines for the upper and lower limits of the confidence interval. In all cases, the estimated curves are fairly close to the true curves, and the confidence intervals include the true curves.

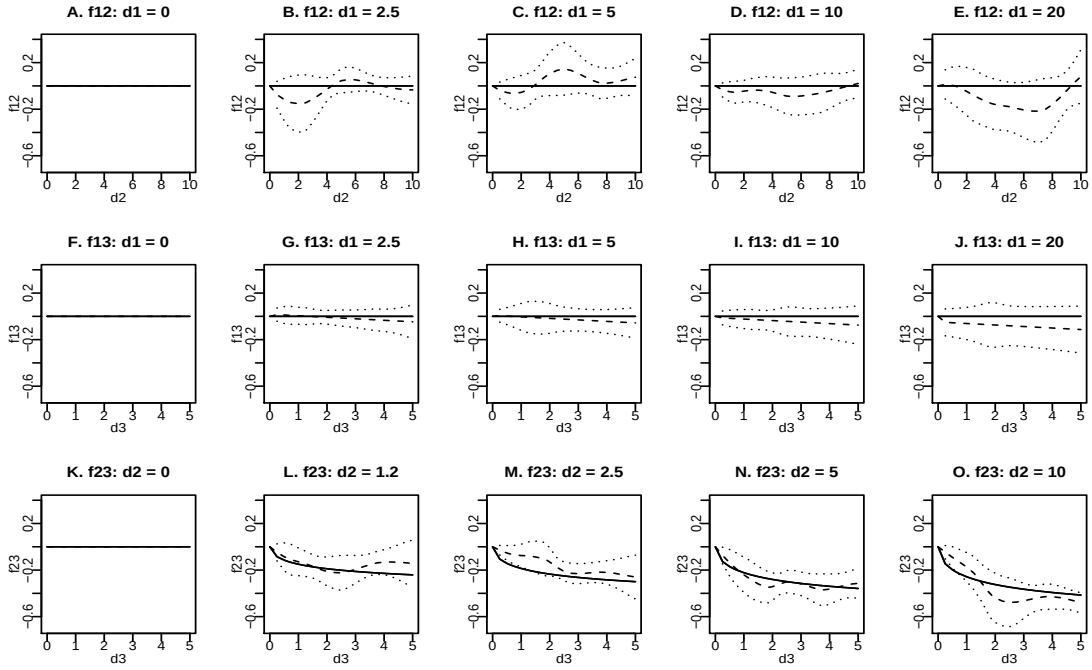

Figure 6.8: Estimated confidence intervals of f_{12}, f_{13} and f_{23} from one of the simulation runs.

Panels F through J of Figure 6.8 show the true and estimated curves of f_{13} for one simulation run (the same simulation run shown in Panels A through F), along with the estimated confidence interval of f_{13}. f_{13} is a function of d_1 and d_3. Each panel shows a cross section of the surface at a different level of d_1, with d_3 plotted on the X-axis, and the value of f_{13} at the corresponding values of d_1 and d_3 plotted on the Y-axis. In each panel, the true curve of f_{13} is shown as a solid black line, the estimated curve of f_{13} from the simulation run is shown as a dashed black line, and the estimated confidence

interval of f_{13} from the simulation run is shown with dotted black lines for the upper and lower limits of the confidence interval. In all cases, the estimated curves are fairly close to the true curves, and the confidence intervals include the true curves.

Panels K through O of Figure 6.8 show the true and estimated curves of f_{23} for one simulation run, along with the estimated confidence interval of f_{23}. f_{23} is a function of d_2 and d_3. Each panel shows a cross section of the surface at a different level of d_2, with d_3 plotted on the X-axis, and the value of f_{23} at the corresponding values of d_2 and d_3 plotted on the Y-axis. In each panel, the true curve of f_{23} is shown as a solid black line, the estimated curve of f_{23} from the simulation run is shown as a dashed black line, and the estimated confidence interval of f_{23} from the simulation run is shown with dotted black lines for the upper and lower limits of the confidence interval. In all cases, the estimated curves are fairly close to the true curves, and the confidence intervals include the true curves.

Figure 6.9 shows the estimated confidence intervals of f_{123} for one of the simulation runs (the same simulation run as in Figure 6.8). f_{123} is a function of three variables, the dose levels of the three drugs, and corresponds to a surface in 4-dimensional space. Like the plots of the model fit previously shown in Figure 6.7, it is shown here as multiple two-dimensional cross sections of the surface, with each panel showing the cross section at different levels of d_1 and d_2. Each panel then shows the cross section with d_3 plotted on the X-axis and the value of f_{123} at the corresponding values of d_1, d_2, and d_3 shown on the Y-axis. In each panel, the true curve of f_{123} is shown as a solid black line, the estimated curve of f_{123} from the simulation run is shown as a dashed black line, and the estimated confidence interval of f_{123} from the simulation run is shown with dotted black lines for the upper and lower limits of the confidence interval. In all cases, the estimated curves are fairly close to the true curves, and the confidence intervals usually include the true curves.

The following graphs evaluate and summarize in more detail the simulation results for the nonparametric functions f_{12}, f_{13}, f_{23}, and f_{123}. Each function is summarized by two graphs; the first graph is a box plot of the bias, percent bias, and confidence interval coverage for the function. The percent bias is the bias divided by the true

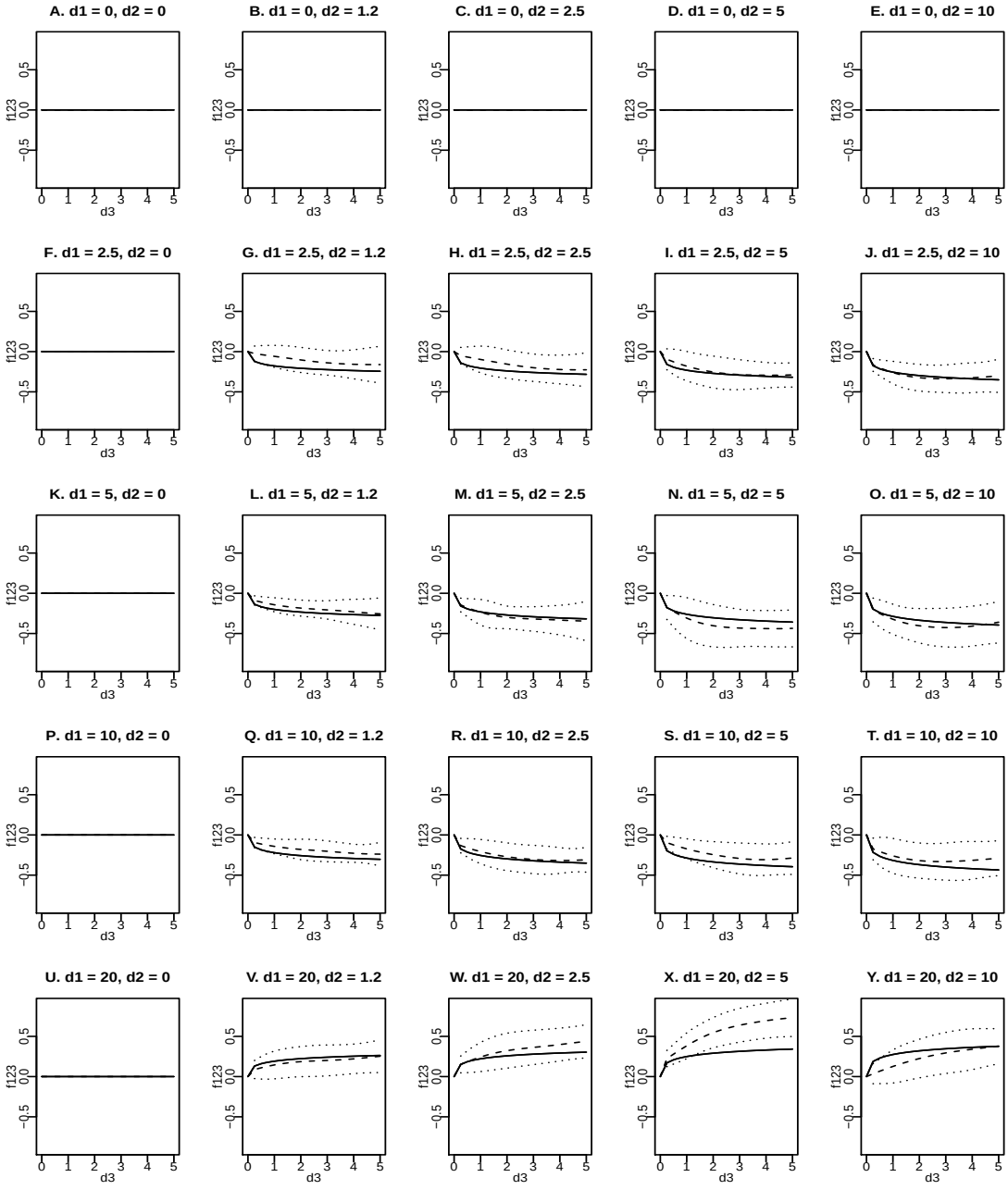

Figure 6.9: Estimated confidence intervals of f_{123} from one of the simulation runs.

value, times 100%; it is not defined if the true value is zero. The confidence interval coverage shows the percentage of time the estimated 95% confidence for the function included its true value.

The second graph shows the percentage of time the function at a particular dose combination was classified as either synergistic, additive, or antagonistic. For the extended Kong and Lee parametric method, it was possible to determine the "correct" classification for each dose combination, and compare the estimated classification to the "correct" classification. But that is not possible to do here for the extended Kong and Lee semiparametric method. The classification of a function at a particular dose combination is based on the confidence interval for the function at that dose combination, not just the estimated value of the function. So although the true value of the function is known, a true value that is close to zero may be classified as additive if it is not sufficiently far from zero.

For the parametric method, it was possible to theoretically determine how far from zero the true function would have to be in order to be considered synergistic or additive. The distance depended on the variability of the model functions, and was a function of the known true standard deviation used in the simulation, and the design matrix of the parametric model. But in this semiparametric method the standard error for the nonparametric functions is estimated using a wild bootstrap and it is not possible to determine what the "expected" or "true" standard error of the function is.

While the following graphs summarize the performance of the method over all dose combinations, further details are available in Appendix D, which lists the performance of the method at each dose combination.

Figures 6.10 and 6.11 summarize the performance for estimating function f_{12}, which models any two-way synergy between drugs 1 and 2. The true value of the function is always zero, so the percent bias is not defined. The absolute bias measurements were very close to zero, and the confidence interval coverage was close to its nominal level of 95%. The function was correctly classified as additive most of the time.

Figures 6.12 and 6.13 summarize the performance for estimating function f_{13}, which models any two-way synergy between drugs 1 and 3. The true value of the function is

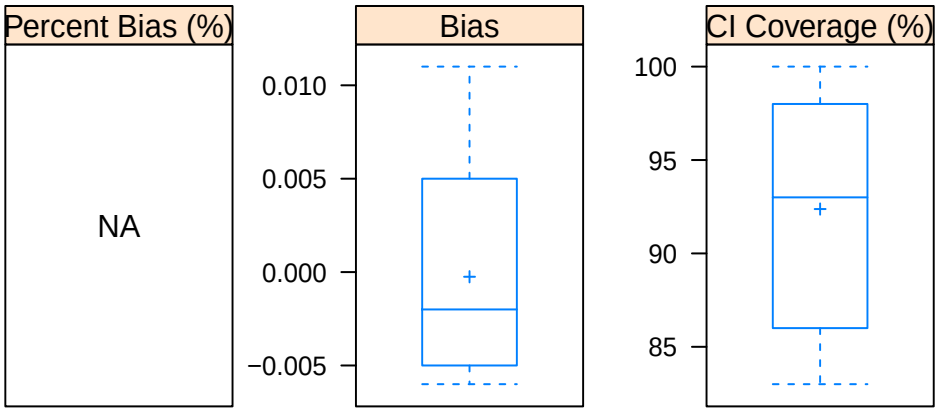

Figure 6.10: Bias and confidence interval coverage for f_{12} in the simulation runs.

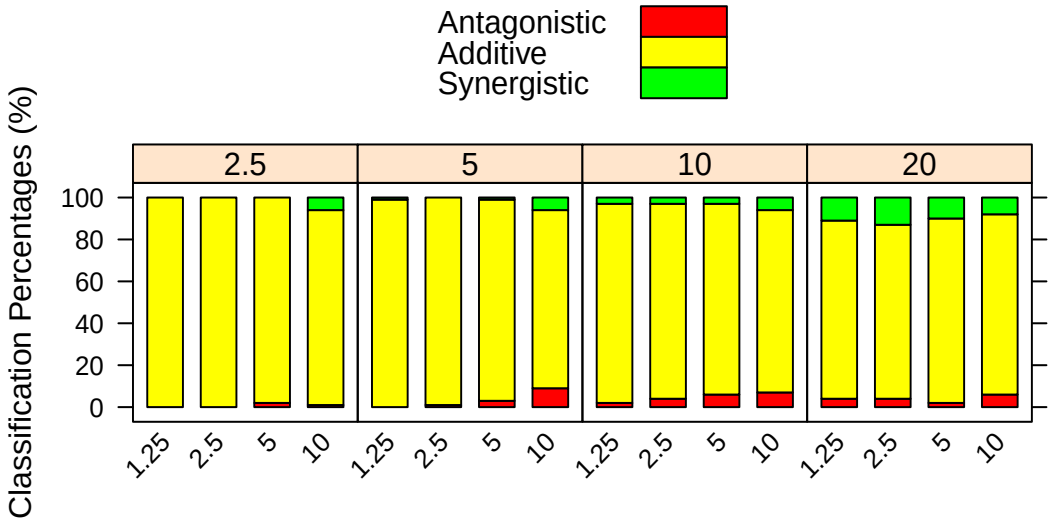

Figure 6.11: Classification percentages for f_{12} in the simulation runs. The shaded labels at the top of each panel indicate the dose of Drug 1, while the diagonal labels along the bottom of the panels are the dose of Drug 2.

always zero, so the percent bias is not defined. The absolute bias measurements were reasonably small. The median confidence interval coverage is around 85%, not 95%. The function was correctly classified as additive most of the time.

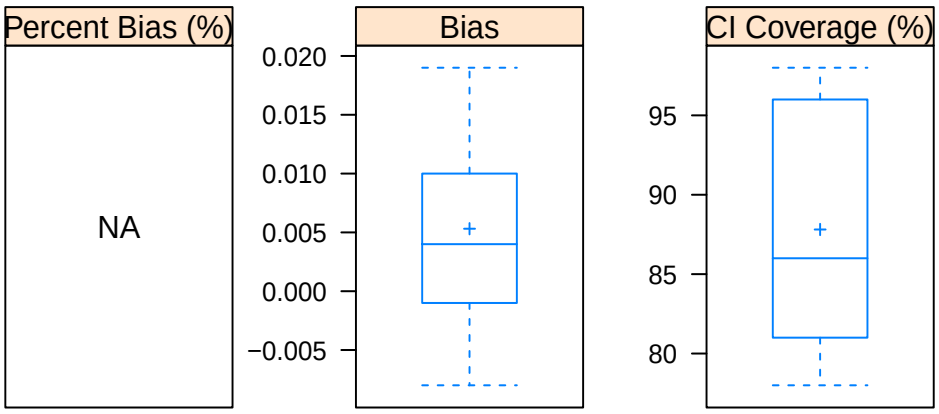

Figure 6.12: Bias and confidence interval coverage for f_{13} in the simulation runs.

Figures 6.14 and 6.15 summarize the performance for estimating function f_{23}, which models any two-way synergy between drugs 2 and 3. The percent bias is not too large, with a median of approximately -10% (the the percent bias and bias have opposite signs because the true value of the function is negative). The median confidence interval coverage is around 75%, not 95%. The function was classified as synergistic most of the time.

Figures 6.16 and 6.17 summarize the performance for estimating function f_{123}, which models any three-way synergy between drugs 1, 2, and 3. The percent bias is not too large, with a median not far from 0%, and the bias also very close to 0. The median confidence interval coverage is around 90%, not 95%. When the dose of Drug 1 was less than 20, the function was classified as synergistic most of the time; when the dose of Drug 1 was equal to 20, the function was classified as antagonistic much of the time.

As the preceding figures have shown, the performance of the method was good in most cases, for estimating the nonparametric functions and their confidence intervals,

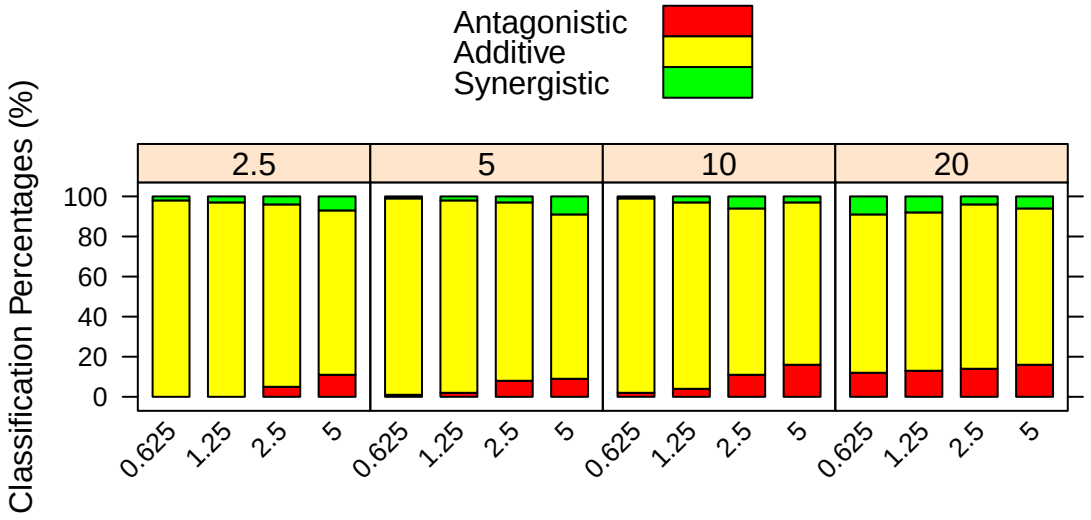

Figure 6.13: Classification percentages for f_{13} in the simulation runs. The shaded labels at the top of each panel indicate the dose of Drug 1, while the diagonal labels along the bottom of the panels are the dose of Drug 3.

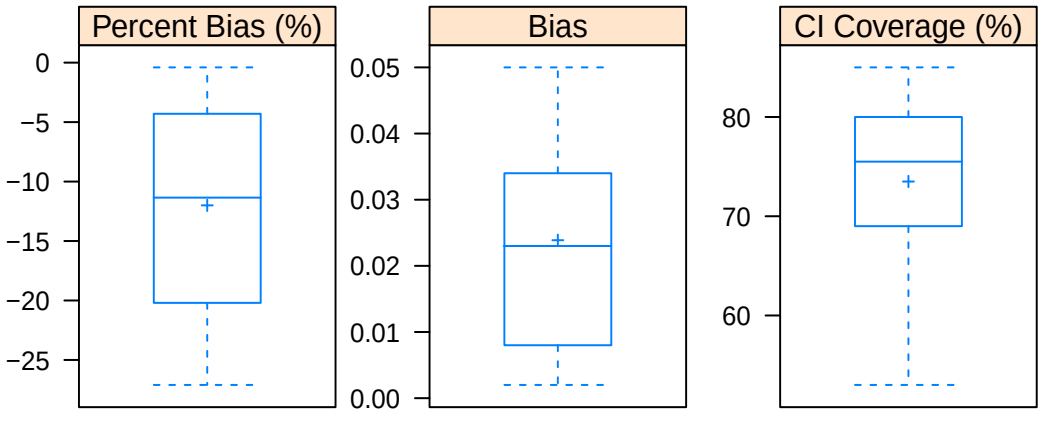

Figure 6.14: Bias and confidence interval coverage for f_{23} in the simulation runs.

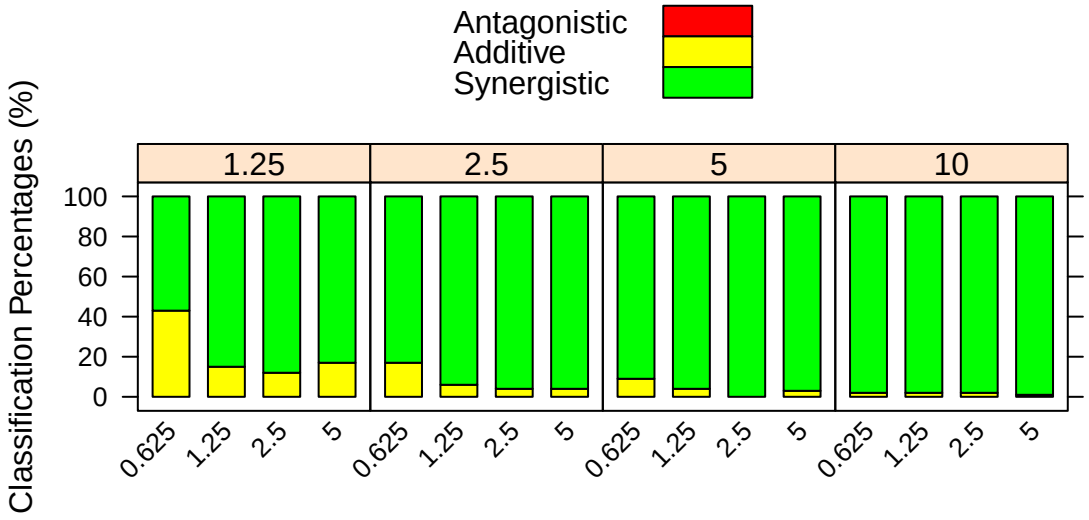

Figure 6.15: Classification percentages for f_{23} in the simulation runs. The shaded labels at the top of each panel indicate the dose of Drug 2, while the diagonal labels along the bottom of the panels are the dose of Drug 3.

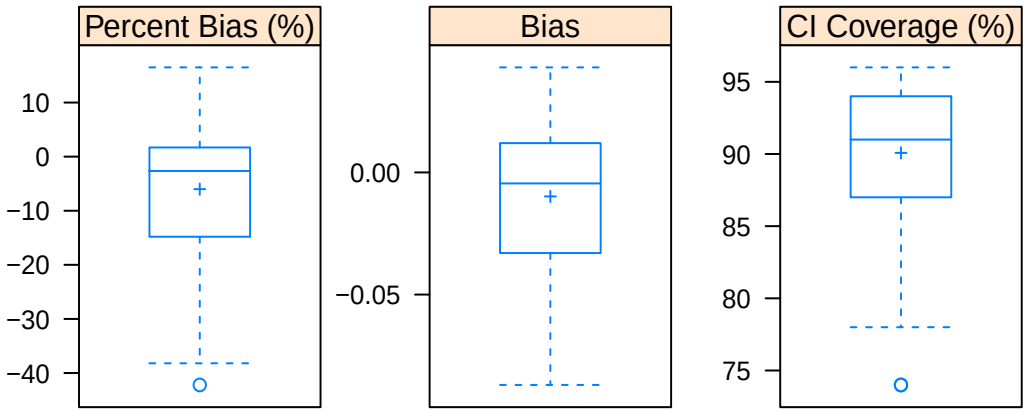

Figure 6.16: Bias and confidence interval coverage for f_{123} in the simulation runs.

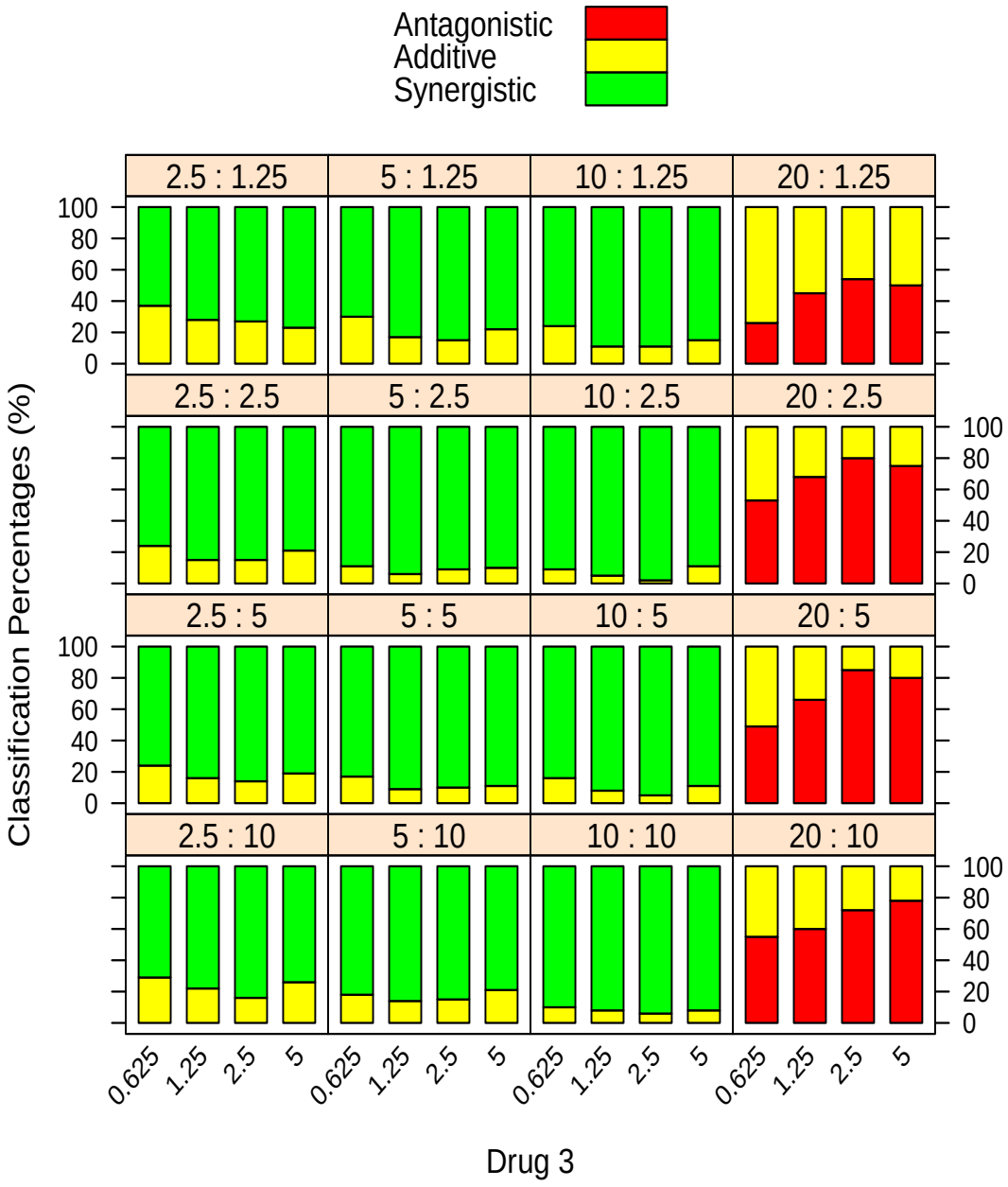

Figure 6.17: Classification percentages for f_{123} in the simulation runs. The shaded labels at the top of each panel indicate the dose of Drug 1 and Drug 2 (as Dose 1 : Dose 2), while the diagonal labels along the bottom of the panels are the dose of Drug 3.

and in identifying cases of synergy or antagonism.

Chapter 7

Summary, Discussion and Future Directions

7.1 Summary

Three response surface methods for determining drug synergy with three or more drugs have been described and evaluated in the previous chapters. The three methods are able to identify increasingly complicated cases of synergy.

With n drugs, there are a total of $\sum_{p=2}^{n} \binom{n}{p}$ interactions between the unique combinations of drugs. Each of the three methods separately models each interaction, but the methods differ in how they model the interactions.

The extended Plummer and Short method is the most limited method in that it assumes any synergy or antagonism between a set of drugs is "global", that is the same at all dose levels. The method uses a single parameter coefficient to model the synergy between a particular combination of drugs, so any synergy is the same at all dose levels of that combination of drugs.

But for interactions that meet the assumptions of the extended Plummer and Short method, it was shown to accurately model the response surface for a number of synergy scenarios, and to provide a good fit of the data.

The only drawback of the method is for interactions that have local synergy, such as the fourth scenario in Section 4.3, which cannot be accurately modeled by the method. However, if the only synergy present is global and not local, the extended Plummer and Short method is the most sensitive of the presented methods, and will require smaller sample sizes to detect the synergy.

The extended Kong and Lee parametric method generalizes the extended Plummer and Short method in order to detect local synergy or antagonism. Where the extended

Plummer and Short method used a single parameter coefficient to model the synergy between a particular combination of drugs, the extended Kong and Lee parametric method uses a function. This function is a function of the doses of the drugs and of a set of parameters that allow the strength and direction of the synergy or antagonism to vary flexibly with the doses. Interactions that have local synergy, such as the second scenario in Section 5.3.3 can be accurately modeled by the extended Kong and Lee parametric method.

The extended Kong and Lee parametric method was shown to accurately estimate the response surfaces for a number of synergy scenarios, including a scenario with local synergy and antagonism; the model also provided a good fit of the data.

The drawback of the extended Kong and Lee parametric method over the Plummer and Short method is the larger sample size that is required to estimate so many additional parameters.

Like the extended Kong and Lee parametric method, the extended Kong and Lee semiparametric method also generalizes the extended Plummer and Short method in order to detect local synergy or antagonism, but it does so differently. Instead of simply replacing the synergy parameters coefficient in the extended Plummer and Short model, the extended Kong and Lee semiparametric model is an additive model that includes an extended Plummer and Short model that has been restricted to assume additivity between the drugs. Nonparametric functions are also included in the additive model to model any departure from additivity. The nonparametric functions are functions of the doses of the drugs, which allows the strength and direction of the synergy or antagonism to vary flexibly with the doses. Interactions that have local synergy, such as the fifth scenario in Section 6.5 can be accurately modeled by the extended Kong and Lee semiparametric method.

The extended Kong and Lee semiparametric method was shown to accurately estimate the response surfaces for a number of synergy scenarios, including a scenario with local synergy and antagonism; the model also provided a good fit of the data.

The increased flexibility of the extended Kong and Lee semiparametric method does come with some drawbacks over the other two methods. The primary drawback is the

larger sample size that is required to estimate the nonparametric functions that model the departure from additivity. The simulation data used in this dissertation used at least twice as many repetitions at each dose combination for the semiparametric method than was used for the parametric method (twice as many repetitions were used for drug combinations used to estimate the 3-way interaction, six times as many were used for drug combinations used to estimate the 2-way interactions). The other drawback of the semiparametric method is that its sequential nature makes it too difficult to estimate its variability based on any theoretical method; the variability can only be estimated using a wild bootstrap, which increases the amount of time required to fit the model.

7.2 Discussion

The motivating example for this dissertation, in Section 1.2, was a synergy study of 4 compounds. However due to some experimental design issues of the study, it was not possible to analyze the study using the any of the methods developed in this dissertation. This section will discuss the design issues and propose an experimental design for a future study that could be analyzed using the methods of this dissertation.

For four compounds the response surface is a surface in 5-dimensional space, with 4 of the dimensions corresponding to the doses of the individual compounds, and the fifth dimension representing the response. Ideally, responses will be available for all possible dose combinations, in order to accurately estimate the response surface. The motivating example did not test all possible dose combinations that would make up a complete grid of points, it only tested a subset of points, which consisted of a sparse grid of points.

Although the compounds in the motivating example were tested individually with between 3 and 6 dose levels, if we assume each drug was only tested at 2 dose levels it is easier to show the sparseness of the grid and to propose a feasible new design of a complete grid of points. For simplicity we also assume the 2 dose levels of each compound are 1 and 2, although the actual levels can be different in the proposed design, and are not important for the structure of the grid.

Figure 7.1 shows the grid of points that make up a complete grid of all possible points for 4 compounds, tested with 2 dose levels each. Each panel of the figure shows the grid of points for a specific dose combination of Drug 1 and Drug 2, whose dose levels are shown in the shaded area immediately above the panel (e.g. the panel in the upper left corner shows the grid of points where the dose of Drug 1 is 0 and the dose of Drug 2 is 2, indicated by the label "Drug 1 = 0, Drug 2 = 2"). Within each panel, the dose of Drug 3 is shown on the X-axis and the dose of Drug 4 is shown on the Y-axis. So the point in the upper left corner of the panel in the upper left corner represents the combination where the dose of Drug 1 is 0 and the dose of Drug 2 is 2, the dose of Drug 3 is 0, and the dose of Drug 4 is 2.

In Figure 7.1, two different symbols are used to distinguish combinations that were tested in the motivating example from combinations that were not tested: the filled-in black circles represent dose combinations there were tested, while the blue circle outlines represent dose combinations that were not tested. This helps illustrate the sparseness of the grid of points tested; ideally each point shown in the grid would have been tested.

In the motivating example, the most critical missing dose combinations in the sparse grid of points tested were the 2-drug combinations that did not include TPA. Although TPA was paired with each other compound for testing, none of the other compounds were paired with each other for testing. Without these combinations it is not possible to test for synergy between those pairs of compounds, and thus not possible to adjust for all of the possible cases of 2-way synergy when assessing any 3-way synergy or 4-way synergy. Although the experiment only tested a small proportion of total dose combinations, if the other pairs of compounds other than TPA had been tested with each other, it would have been possible to analyze the results using one of the methods developed in this dissertation. Because only one dose combination was tested for each 3-way interaction and for the 4-way interaction, it would not be possible to test for local synergy so global synergy would have to be assumed. Thus the extended Plummer and Short model would be the most appropriate method to use for the analysis, although the extended Kong and Lee semiparametric method could be used if the assumption of individual log-dose response curves was not met.

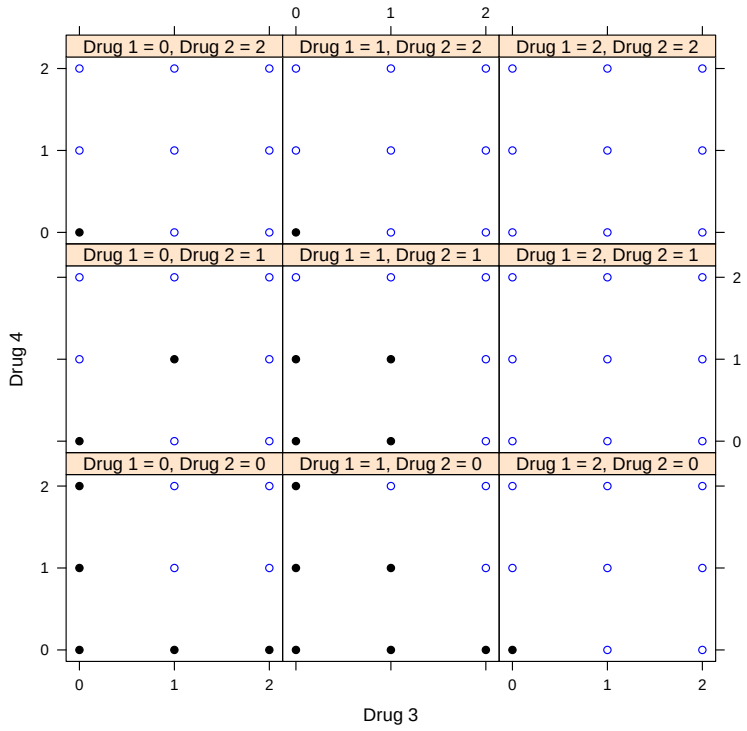

Figure 7.1: Dose combinations tested in the motivating example.

In order to more thoroughly analyze the synergy properties of the 4 compounds tested in the motivating example, it would be necessary to re-do the experiment using a different design. One feasible design would be to use only two dose levels for all four compounds, but test all unique combinations of the compounds. If this design were shown in a graph like that in Figure 7.1, each point of the graph would be a filled-in black circle. With two dose levels, and the doses of zero for testing for lower-order interactions, the proposed design would require testing $(2+1)^4 = 3^4 = 81$ dose combinations. With this design it would then be possible to identify any 2-way, 3-way and 4-way synergy between the compounds.

An even simpler design could be used for the motivating example if the dose of interest for one of the drugs, such as TPA, has already been determined, and the goal of the study is to identify synergy between the other drugs, in the presence of that drug. In that case, the design reduces to a synergy study of only 3 drugs. With two dose levels, and the doses of zero for testing for lower-order interactions, a design would require test testing only $(2+1)^3 = 3^3 = 27$ dose combinations. With this design it would then be possible to identify any 2-way and 3-way synergy between the three compounds, in the presence of the first compound. If this design were shown in a graph like that in Figure 7.1, only one of the three columns of panels would be needed (e.g. the center column of panels, where the dose of Drug 1 is always equal to 1), and only the points of the three panels in that column of the graph would have to be filled-in black circles.

7.3 Future Directions

The previous chapters described a number of new approaches to modeling synergy between three or more drugs, but there are still areas where additional work could be done.

7.3.1 Generalize Loewe additivity model

In Chapter 3 the Loewe additivity model was extended to accommodate three or more drugs. But the extended model can only identify whether the overall effects are synergistic, antagonistic or additive; it cannot distinguish between n-way synergy, and p-way synergy, $2 \leq p < n$, and it cannot take into account more than one synergistic relationship between the different combinations of drugs. At a minimum, the model should be generalized so it can take into account and adjust for any lower order synergy. Ideally, a generalized model should be created that simultaneously models all interactions between all combinations of drugs.

7.3.2 Find most synergistic combination of drugs

Another area for future work is to find the most synergistic combination for a given total dose of drugs. Focusing on the 2-drug case, for simplicity, assume the two drugs are given in doses d_1 and d_2 respectively, for a total dose of $d_1 + \rho d_2 = d_t$, and that the total dose of d_t is fixed, and that D_1 is the dose of drug 1 alone that has the same response as the combination, and that D_2 is the dose of drug 2 alone that has the same response as the combination. If the drugs are additive, then the points $(D_1, 0)$ and $(0, D_2)$ are connected by a line, and all points (d_1, d_2) along that line have the same response. But if there is synergy, then some of the points have a better response, and it would be beneficial to find the pair of points with the best response.

7.3.3 Improve extended Kong and Lee parametric method model selection

The extended Kong and Lee parametric method described Chapter 5 used a parametric model to model a complicated response surface for interactions between combinations of drugs. The model included a large number of parameters, but only some of them may be significant. The method used a backward elimination procedure to remove unnecessary parameters from the model, as had been done in Kong and Lee's parametric method for two drugs. But with more than two drugs the backward elimination procedure did not

work very well, and the full model generally performed better than the final, reduced model. Another area of work would be to find a better method of model selection, perhaps one as simple as a forward selection method, or something more complicated such as LASSO or ridge regression.

7.3.4 Use nonparametric functions in extended Kong and Lee parametric method

The extended Kong and Lee parametric method used parametric functions to flexibly model the synergy relationships. Alternatively, nonparametric functions could be used. More specifically, this proposed model would keep the overall structure of the extended Kong and Lee parametric model, specified in (5.1), but replace the parametric functions specified in (5.2) with nonparametric functions.

This approach would allow even greater flexibility than the current parametric functions. And by using the nonparametric functions in the context of the parametric model, but without switching to the multi-stage estimation used in the extended Kong and Lee semiparametric model, it would not require the use of the wild bootstrap for variance estimation.

7.3.5 Experimental design and sample size

And finally, two other related areas for future work are in experimental design and sample size. Finding an optimal design for an experiment, and determining the sample size requirements would be useful for all of the extended methods, but especially for the extended Kong and Lee semiparametric method described in Chapter 6. The semiparametric method sequentially estimates the different interactions, starting from the 2-way interactions and proceeding in increasing order to the n-way interaction. This makes it critical that the lower order interactions are estimated accurately, so they do not bias the estimations of the higher order interactions. Chapter 6 used larger sample sizes to estimate the lower order interactions (the "edges" of the design space, where one or more of the drugs has a dose of 0), but additional work could determine the optimal ratio of the sample sizes for the drug combinations for the different order interactions.

Appendix A

Extended Kong and Lee Parametric Method Model Function Derivatives

The extended Kong and Lee parametric method is shown below:

$$
\begin{aligned}
Y = \beta_0 + \beta_1 \log(d_1 + \rho_2 d_2 + \rho_3 d_3 \\
&+ f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_{12}\right)\left(d_1 \rho_2 d_2\right)^{1/2} + f_{13}\left(d_1, d_3; \beta_4, \beta 5, \kappa_{13}\right)\left(d_1 \rho_3 d_3\right)^{1/2} \\
&+ f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right)\left(\rho_2 d_2 \rho_3 d_3\right)^{1/2} \\
&+ f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right)\left(d_1 \rho_2 d_2 \rho_3 d_3\right)^{1/3})
\end{aligned}
$$

where:

$$
\begin{aligned}
\log\left(\rho_2\right) &= \beta_2 + \beta_3 \log\left(D_2\right) \\
D_2 &= d_2 + d_1 e^{-\beta_2 - \beta_3 \log(D_2)} = d_2 + d_1/\rho_2
\end{aligned}
$$

$$
\begin{aligned}
\log\left(\rho_3\right) &= \beta_4 + \beta_5 \log\left(D_3\right) \\
D_3 &= d_3 + d_1 e^{-\beta_4 - \beta_5 \log(D_3)} = d_3 + d_1/\rho_3
\end{aligned}
$$

and

$$
\begin{aligned}
f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_{12}\right) =& \kappa_{12,0} + \kappa 12, 1 d_1^{\frac{1}{2}} + \kappa_{12,2}\left(\rho_2 d_2\right)^{\frac{1}{2}} + \kappa_{12,3} d_1 \\
&+ \kappa 12, 4 \rho_2 d_2 + \kappa_{12,5}\left(d_1 \rho_2 d_2\right)^{\frac{1}{2}}
\end{aligned}
$$

$$
\begin{aligned}
f_{13}\left(d_1, d_3; \beta_4, \beta_5, \kappa_{13}\right) =& \kappa_{13,0} + \kappa_{13,1} d_1^{\frac{1}{2}} + \kappa_{13,2}\left(\rho_3 d_3\right)^{\frac{1}{2}} + \kappa_{13,3} d_1 \\
&+ \kappa_{13,4} \rho_3 d_3 + \kappa_{13,5}\left(d_1 \rho_3 d_3\right)^{\frac{1}{2}}
\end{aligned}
$$

$$
\begin{aligned}
f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right) =& \kappa_{23,0} + \kappa_{23,1}\left(\rho_2 d_2\right)^{\frac{1}{2}} + \kappa_{23,2}\left(\rho_3 d_3\right)^{\frac{1}{2}} + \kappa_{23,3} \rho_2 d_2 \\
&+ \kappa_{23,4} \rho_3 d_3 + \kappa_{23,5}\left(\rho_2 d_2 \rho_3 d_3\right)^{\frac{1}{2}}
\end{aligned}
$$

$$\begin{aligned} f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right) =& \kappa_{123,0} + \kappa_{123,1} d_1^{\frac{1}{3}} + \kappa_{123,2}\left(\rho_2 d_2\right)^{\frac{1}{3}} + \kappa_{123,3}\left(\rho_3 d_3\right)^{\frac{1}{3}} \\ &+ \kappa_{123,4} d_1 + \kappa_{123,5} \rho_2 d_2 + \kappa_{123,6} \rho_3 d_3 \\ &+ \kappa_{123,7}\left(d_1 \rho_2 d_2 \rho_3 d_3\right)^{\frac{1}{3}} \end{aligned}$$

The parameters modeling the relative potency of the drugs to each other can be restated as:

$$\log\left(\rho_2\right) = \beta_2 + \beta_3 \log\left(D_2\right)$$

$$\rho_2 = e^{\beta_2 + \beta_3 \log(D_2)}$$

$$\log\left(\rho_3\right) = \beta_4 + \beta_5 \log\left(D_3\right)$$

$$\rho_3 = e^{\beta_4 + \beta_5 \log(D_3)}$$

and then the model itself can be restated equivalently as:

$$\begin{aligned} Y = \beta_0 + \beta_1 \log\Bigg(& d_1 + d_2 e^{\beta_2 + \beta_3 \log(D_2)} + d_3 e^{\beta_4 + \beta_5 \log(D_3)} \\ & + f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_{12}\right)\left(d_1 d_2 e^{\beta_2 + \beta_3 \log(D_2)}\right)^{1/2} \\ & + f_{13}\left(d_1, d_3; \beta_4, \beta 5, \kappa_{13}\right)\left(d_1 d_3 e^{\beta_4 + \beta_5 \log(D_3)}\right)^{1/2} \\ & + f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right)\left(d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)}\right)^{1/2} \\ & + f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right) \\ & \times\left(d_1 d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)}\right)^{1/3}\Bigg) \end{aligned}$$

The extended Kong and Lee parametric model is a nonlinear model. Nonlinear models in general are of the form:

$$Y_i = f\left(X_i, \gamma\right) + \epsilon_i$$

where X_i is the vector of predictor variables for the i^{th} case:

$$X_i = \begin{bmatrix} X_{i1} \\ X_{i2} \\ \cdots \\ X_{iq} \end{bmatrix}$$

and γ is the vector of regression coefficients:

$$\gamma = \begin{bmatrix} \gamma_0 \\ \gamma_1 \\ \cdots \\ \gamma_{p-1} \end{bmatrix}$$

Nonlinear models are generally fitted using numerical search procedures to estimate the regression parameters and their standard errors. One common method is the Gauss-Newton method (see [25] for additional details). The Gauss-Newton method iteratively estimates the regression parameter vector γ with a vector g:

$$g = \begin{bmatrix} g_0 \\ g_1 \\ \cdots \\ g_{p-1} \end{bmatrix}$$

using repeated approximations based on a Taylor series expansion of the function f. The Taylor series expansion is calculated using a matrix of partial derivatives of the function f with respect to the parameters γ:

$$D_{ik} = \left[\frac{\partial f\left(X_i, \gamma\right)}{\partial \gamma_k} \right]_{\gamma = g} \tag{A.1}$$

Once the parameters have been estimated, the D matrix is also used to estimate the standard errors of the parameter estimates, using the estimated error term:

$$s^2\left\{g\right\} = MSE\left(D'D\right)^{\prime -1} \tag{A.2}$$

In the extended Kong and Lee parametric method in Chapter 5, the functions f_{12}, f_{13}, f_{23} and f_{123} at a given dose combination are classified as either synergistic, antagonistic or additive. This classification depends on the estimated standard errors of the functions, which are determined using the delta method and the estimated standard errors of the model parameters; the estimated standard errors of the model parameters are estimated as described above.

To determine the "correct" classification of the functions in order to evaluate the performance of the method, the "true standard error" of each parameter is calculated

using (A.2), but using the simulations' known true standard deviation and known true parameter values. This calculation depends on the D matrix in (A.1). The derivatives for the D matrix of the extended Kong and Lee parametric model for three drugs are shown below.

For the extended Kong and Lee parametric method the function f of the nonlinear model is defined as:

$$\begin{aligned} f = \beta_0 + \beta_1 \log \Bigg(& d_1 + d_2 e^{\beta_2 + \beta_3 \log(D_2)} + d_3 e^{\beta_4 + \beta_5 \log(D_3)} \\ & + f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_{12}\right) \left(d_1 d_2 e^{\beta_2 + \beta_3 \log(D_2)}\right)^{1/2} \\ & + f_{13}\left(d_1, d_3; \beta_4, \beta 5, \kappa_{13}\right) \left(d_1 d_3 e^{\beta_4 + \beta_5 \log(D_3)}\right)^{1/2} \\ & + f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right) \left(d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)}\right)^{1/2} \\ & + f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right) \\ & \times \left(d_1 d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)}\right)^{1/3} \Bigg) \end{aligned}$$

and the parameter vector $\gamma = (\beta_0, \beta_1, \beta_2,\ \beta_3, \beta_4, \beta_5,\ \kappa_{12,0}, \ldots, \kappa_{12,5},\ \kappa_{13,0}, \ldots, \kappa_{13,5},\ \kappa_{23,0}, \ldots, \kappa_{23,5},\ \kappa_{123,0}, \ldots, \kappa_{123,6}, \kappa_{123,7})'$.

As a notational convenience, Λ will be defined to be as:

$$\begin{aligned} \Lambda = & d_1 + d_2 e^{\beta_2 + \beta_3 \log(D_2)} + d_3 e^{\beta_4 + \beta_5 \log(D_3)} \\ & + f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_{12}\right) \left(d_1 d_2 e^{\beta_2 + \beta_3 \log(D_2)}\right)^{1/2} \\ & + f_{13}\left(d_1, d_3; \beta_4, \beta 5, \kappa_{13}\right) \left(d_1 d_3 e^{\beta_4 + \beta_5 \log(D_3)}\right)^{1/2} \\ & + f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right) \left(d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)}\right)^{1/2} \\ & + f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right) \left(d_1 d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)}\right)^{1/3} \end{aligned}$$

The derivatives that are needed for the D matrix can then be defined using:

$$\frac{\partial f}{\partial \beta_0} = 1$$

$$\frac{\partial f}{\partial \beta_1} = \log\left(\Lambda\right)$$

$$\frac{\partial f}{\partial \beta_2} = \frac{\beta_1}{\Lambda}\Bigg[d_2 e^{\beta_2+\beta_3\log(D_2)} + \frac{1}{2} f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_{12}\right)\left(d_1 d_2 e^{\beta_2+\beta_3\log(D_2)}\right)^{1/2} + \left(d_1 d_2 e^{\beta_2+\beta_3\log(D_2)}\right)^{1/2}\frac{\partial f_{12}}{\partial \beta_2} + \frac{1}{2} f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right)\left(d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/2} + \left(d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/2}\frac{\partial f_{23}}{\partial \beta_2} + \frac{1}{3} f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right)\left(d_1 d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/3} + \left(d_1 d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/3}\frac{\partial f_{123}}{\partial \beta_2}\Bigg]$$

$$\frac{\partial f}{\partial \beta_3} = \frac{\beta_1}{\Lambda}\Bigg[d_2 e^{\beta_2+\beta_3\log(D_2)}\log\left(D_2\right) + \frac{1}{2} f_{12}\left(d_1, d_2; \beta_2, \beta_3, \kappa_{12}\right)\left(d_1 d_2 e^{\beta_2+\beta_3\log(D_2)}\right)^{1/2}\log\left(D_2\right) + \left(d_1 d_2 e^{\beta_2+\beta_3\log(D_2)}\right)^{1/2}\frac{\partial f_{12}}{\partial \beta_3} + \frac{1}{2} f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right)\left(d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/2}\log\left(D_2\right) + \left(d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/2}\frac{\partial f_{23}}{\partial \beta_3} + \frac{1}{3} f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right)\left(d_1 d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/3} \times \log\left(D_2\right) + \left(d_1 d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/3}\frac{\partial f_{123}}{\partial \beta_3}\Bigg]$$

$$\frac{\partial f}{\partial \beta_4} = \frac{\beta_1}{\Lambda}\Bigg[d_3 e^{\beta_4+\beta_5\log(D_3)} + \frac{1}{2} f_{13}\left(d_1, d_3; \beta_4, \beta_5, \kappa_{13}\right)\left(d_1 d_3 e^{\beta_4+\beta_5\log(D_3)}\right)^{1/2} + \left(d_1 d_3 e^{\beta_4+\beta_5\log(D_3)}\right)^{1/2}\frac{\partial f_{13}}{\partial \beta_4} + \frac{1}{2} f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right)\left(d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/2} + \left(d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/2}\frac{\partial f_{23}}{\partial \beta_4} + \frac{1}{3} f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right)\left(d_1 d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/3} + \left(d_1 d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/3}\frac{\partial f_{123}}{\partial \beta_4}\Bigg]$$

$$\begin{aligned}\frac{\partial f}{\partial \beta_5} = \frac{\beta_1}{\Lambda}\Bigg[& d_3 e^{\beta_4+\beta_5\log(D_3)}\log(D_3) \\ & + \frac{1}{2} f_{13}\left(d_1, d_3; \beta_4, \beta_5, \kappa_{13}\right)\left(d_1 d_3 e^{\beta_4+\beta_5\log(D_3)}\right)^{1/2}\log(D_3) \\ & + \left(d_1 d_3 e^{\beta_4+\beta_5\log(D_3)}\right)^{1/2}\frac{\partial f_{13}}{\partial \beta_5} \\ & + \frac{1}{2} f_{23}\left(d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{23}\right)\left(d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/2}\log(D_3) \\ & + \left(d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/2}\frac{\partial f_{23}}{\partial \beta_5} \\ & + \frac{1}{3} f_{123}\left(d_1, d_2, d_3; \beta_2, \beta_3, \beta_4, \beta_5, \kappa_{123}\right)\left(d_1 d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/3} \\ & \times \log(D_3) \\ & + \left(d_1 d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/3}\frac{\partial f_{123}}{\partial \beta_5}\Bigg]\end{aligned}$$

$$\frac{\partial f}{\partial \kappa_{12,h}} = \frac{\beta_1}{\Lambda}\left(d_1 d_2 e^{\beta_2+\beta_3\log(D_2)}\right)^{1/2}\frac{\partial f_{12}}{\partial \kappa_{12,h}} \qquad h \in \{0,1,2,3,4,5\}$$

$$\frac{\partial f}{\partial \kappa_{13,i}} = \frac{\beta_1}{\Lambda}\left(d_1 d_3 e^{\beta_4+\beta_5\log(D_3)}\right)^{1/2}\frac{\partial f_{13}}{\partial \kappa_{13,i}} \qquad i \in \{0,1,2,3,4,5\}$$

$$\frac{\partial f}{\partial \kappa_{23,j}} = \frac{\beta_1}{\Lambda}\left(d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/2}\frac{\partial f_{23}}{\partial \kappa_{23,j}} \qquad j \in \{0,1,2,3,4,5\}$$

$$\frac{\partial f}{\partial \kappa_{123,k}} = \frac{\beta_1}{\Lambda}\left(d_1 d_2 d_3 e^{\beta_2+\beta_3\log(D_2)+\beta_4+\beta_5\log(D_3)}\right)^{1/3}\frac{\partial f_{123}}{\partial \kappa_{123,k}} \quad k \in \{0,1,2,3,4,5,6,7\}$$

$$\frac{\partial f_{12}}{\partial \beta_2} = \frac{1}{2}\kappa_{12,2}\left(d_2 e^{\beta_2+\beta_3\log(D_2)}\right)^{1/2} + \kappa 12, 4 d_2 e^{\beta_2+\beta_3\log(D_2)} + \frac{1}{2}\kappa_{12,5}\left(d_1 d_2 e^{\beta_2+\beta_3\log(D_2)}\right)^{1/2}$$

$$\begin{aligned}\frac{\partial f_{12}}{\partial \beta_3} = & \frac{1}{2}\kappa_{12,2}\left(d_2 e^{\beta_2+\beta_3\log(D_2)}\right)^{1/2}\log(D_2) + \kappa 12, 4 d_2 e^{\beta_2+\beta_3\log(D_2)}\log(D_2) \\ & + \frac{1}{2}\kappa_{12,5}\left(d_1 d_2 e^{\beta_2+\beta_3\log(D_2)}\right)^{1/2}\log(D_2)\end{aligned}$$

$$\frac{\partial f_{12}}{\partial \kappa_{12,0}} = 1$$

$$\frac{\partial f_{12}}{\partial \kappa 12,1} = d_1^{1/2}$$

$$\frac{\partial f_{12}}{\partial \kappa_{12,2}} = \left(d_2 e^{\beta_2+\beta_3 \log(D_2)}\right)^{1/2}$$

$$\frac{\partial f_{12}}{\partial \kappa_{12,3}} = d_1$$

$$\frac{\partial f_{12}}{\partial \kappa 12,4} = d_2 e^{\beta_2+\beta_3 \log(D_2)}$$

$$\frac{\partial f_{12}}{\partial \kappa_{12,5}} = \left(d_1 d_2 e^{\beta_2+\beta_3 \log(D_2)}\right)^{1/2}$$

$$\frac{\partial f_{13}}{\partial \beta_4} = \frac{1}{2}\kappa_{13,2}\left(d_3 e^{\beta_4+\beta_5 \log(D_3)}\right)^{1/2} + \kappa_{13,4} d_3 e^{\beta_4+\beta_5 \log(D_3)} + \frac{1}{2}\kappa_{13,5}\left(d_1 d_3 e^{\beta_4+\beta_5 \log(D_3)}\right)^{1/2}$$

$$\begin{aligned}\frac{\partial f_{13}}{\partial \beta_5} =& \frac{1}{2}\kappa_{13,2}\left(d_3 e^{\beta_4+\beta_5 \log(D_3)}\right)^{1/2}\log(D_3) + \kappa_{13,4} d_3 e^{\beta_4+\beta_5 \log(D_3)}\log(D_3) \\ &+ \frac{1}{2}\kappa_{13,5}\left(d_1 d_3 e^{\beta_4+\beta_5 \log(D_3)}\right)^{1/2}\log(D_3)\end{aligned}$$

$$\frac{\partial f_{13}}{\partial \kappa_{13,0}} = 1$$

$$\frac{\partial f_{13}}{\partial \kappa_{13,1}} = d_1^{1/2}$$

$$\frac{\partial f_{13}}{\partial \kappa_{13,2}} = \left(d_3 e^{\beta_4+\beta_5 \log(D_3)}\right)^{1/2}$$

$$\frac{\partial f_{13}}{\partial \kappa_{13,3}} = d_1$$

$$\frac{\partial f_{13}}{\partial \kappa_{13,4}} = d_3 e^{\beta_4 + \beta_5 \log(D_3)}$$

$$\frac{\partial f_{13}}{\partial \kappa_{13,5}} = \left(d_1 d_3 e^{\beta_4 + \beta_5 \log(D_3)} \right)^{1/2}$$

$$\begin{aligned}\frac{\partial f_{23}}{\partial \beta_2} =& \frac{1}{2}\kappa_{23,1} \left(d_2 e^{\beta_2 + \beta_3 \log(D_2)} \right)^{1/2} + \kappa_{23,3} d_2 e^{\beta_2 + \beta_3 \log(D_2)} \\ &+ \frac{1}{2}\kappa_{23,5} \left(d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)} \right)^{1/2}\end{aligned}$$

$$\begin{aligned}\frac{\partial f_{23}}{\partial \beta_3} =& \frac{1}{2}\kappa_{23,1} \left(d_2 e^{\beta_2 + \beta_3 \log(D_2)} \right)^{1/2} \log(D_2) + \kappa_{23,3} d_2 e^{\beta_2 + \beta_3 \log(D_2)} \log(D_2) \\ &+ \frac{1}{2}\kappa_{23,5} \left(d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)} \right)^{1/2} \log(D_2)\end{aligned}$$

$$\begin{aligned}\frac{\partial f_{23}}{\partial \beta_4} =& \frac{1}{2}\kappa_{23,2} \left(d_3 e^{\beta_4 + \beta_5 \log(D_3)} \right)^{1/2} + \kappa_{23,4} d_3 e^{\beta_4 + \beta_5 \log(D_3)} \\ &+ \frac{1}{2}\kappa_{23,5} \left(d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)} \right)^{1/2}\end{aligned}$$

$$\begin{aligned}\frac{\partial f_{23}}{\partial \beta_5} =& \frac{1}{2}\kappa_{23,2} \left(d_3 e^{\beta_4 + \beta_5 \log(D_3)} \right)^{1/2} \log(D_3) + \kappa_{23,4} d_3 e^{\beta_4 + \beta_5 \log(D_3)} \log(D_3) \\ &+ \frac{1}{2}\kappa_{23,5} \left(d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)} \right)^{1/2} \log(D_3)\end{aligned}$$

$$\frac{\partial f_{23}}{\partial \kappa_{23,0}} = 1$$

$$\frac{\partial f_{23}}{\partial \kappa_{23,1}} = \left(d_2 e^{\beta_2 + \beta_3 \log(D_2)} \right)^{1/2}$$

$$\frac{\partial f_{23}}{\partial \kappa_{23,2}} = \left(d_3 e^{\beta_4 + \beta_5 \log(D_3)} \right)^{1/2}$$

$$\frac{\partial f_{23}}{\partial \kappa_{23,3}} = d_2 e^{\beta_2 + \beta_3 \log(D_2)}$$

$$\frac{\partial f_{23}}{\partial \kappa_{23,4}} = d_3 e^{\beta_4 + \beta_5 \log(D_3)}$$

$$\frac{\partial f_{23}}{\partial \kappa_{23,5}} = \left(d_2 d_3 e^{\beta_2+\beta_3 \log(D_2)+\beta_4+\beta_5 \log(D_3)}\right)^{1/2}$$

$$\begin{aligned}\frac{\partial f_{123}}{\partial \beta_2} =& \frac{1}{3}\kappa_{123,2}\left(d_2 e^{\beta_2+\beta_3 \log(D_2)}\right)^{1/3} + \kappa_{123,5} d_2 e^{\beta_2+\beta_3 \log(D_2)} \\ &+ \frac{1}{3}\kappa_{123,7}\left(d_1 d_2 d_3 e^{\beta_2+\beta_3 \log(D_2)+\beta_4+\beta_5 \log(D_3)}\right)^{1/3}\end{aligned}$$

$$\begin{aligned}\frac{\partial f_{123}}{\partial \beta_3} =& \frac{1}{3}\kappa_{123,2}\left(d_2 e^{\beta_2+\beta_3 \log(D_2)}\right)^{1/3}\log(D_2) + \kappa_{123,5} d_2 e^{\beta_2+\beta_3 \log(D_2)}\log(D_2) \\ &+ \frac{1}{3}\kappa_{123,7}\left(d_1 d_2 d_3 e^{\beta_2+\beta_3 \log(D_2)+\beta_4+\beta_5 \log(D_3)}\right)^{1/3}\log(D_2)\end{aligned}$$

$$\begin{aligned}\frac{\partial f_{123}}{\partial \beta_4} =& \frac{1}{3}\kappa_{123,3}\left(d_3 e^{\beta_4+\beta_5 \log(D_3)}\right)^{1/3} + \kappa_{123,6} d_3 e^{\beta_4+\beta_5 \log(D_3)} \\ &+ \frac{1}{3}\kappa_{123,7}\left(d_1 d_2 d_3 e^{\beta_2+\beta_3 \log(D_2)+\beta_4+\beta_5 \log(D_3)}\right)^{1/3}\end{aligned}$$

$$\begin{aligned}\frac{\partial f_{123}}{\partial \beta_5} =& \frac{1}{3}\kappa_{123,3}\left(d_3 e^{\beta_4+\beta_5 \log(D_3)}\right)^{1/3}\log(D_3) + \kappa_{123,6} d_3 e^{\beta_4+\beta_5 \log(D_3)}\log(D_3) \\ &+ \frac{1}{3}\kappa_{123,7}\left(d_1 d_2 d_3 e^{\beta_2+\beta_3 \log(D_2)+\beta_4+\beta_5 \log(D_3)}\right)^{1/3}\log(D_3)\end{aligned}$$

$$\frac{\partial f_{123}}{\partial \kappa_{123,0}} = 1$$

$$\frac{\partial f_{123}}{\partial \kappa_{123,1}} = d_1^{1/3}$$

$$\frac{\partial f_{123}}{\partial \kappa_{123,2}} = \left(d_2 e^{\beta_2+\beta_3 \log(D_2)}\right)^{1/3}$$

$$\frac{\partial f_{123}}{\partial \kappa_{123,3}} = \left(d_3 e^{\beta_4+\beta_5 \log(D_3)}\right)^{1/3}$$

$$\frac{\partial f_{123}}{\partial \kappa_{123,4}} = d_1$$

$$\frac{\partial f_{123}}{\partial \kappa_{123,5}} = d_2 e^{\beta_2+\beta_3 \log(D_2)}$$

$$\frac{\partial f_{123}}{\partial \kappa_{123,6}} = d_3 e^{\beta_4 + \beta_5 \log(D_3)}$$

$$\frac{\partial f_{123}}{\partial \kappa_{123,7}} = \left(d_1 d_2 d_3 e^{\beta_2 + \beta_3 \log(D_2) + \beta_4 + \beta_5 \log(D_3)} \right)^{1/3}$$

Appendix B

Extended Kong and Lee Parametric Method Model Function Goodness of Fit Tables

In Section 5.4.2, the extended Kong and Lee parametric method evaluated the performance of the method by evaluating the estimation of the model functions, f_{12}, f_{13}, f_{23} and f_{123} at each dose combination. Because of the large number of dose combinations, particularly for the f_{123} function, the results of the evaluation were summarized graphically in that section. The following tables show the individual results at each dose combination, under each of the two scenarios evaluated, for both the full model and the final model.

The tables columns are labeled as follows; see Section 5.4.2 for additional details.

true the true value of the function.

ave the mean of the estimated values of the function.

abs.bias the bias.

rel.bias the percent bias, divided by 100%.

mse the mean squared error of the estimates.

cr.ci the confidence interval coverage,divided by 100%.

p.ant the percentage of times the function was classified as antagonistic.

p.add the percentage of times the function was classified as additive.

p.syn the percentage of times the function was classified as synergistic.

B.1 Model Function Goodness of Fit Tables, First Scenario

B.1.1 Full Model

Table B.1 shows the detailed results from the evaluation of f_{12}, based on the full model. The function has a constant true value of 0.4, indicating antagonism, and this is correctly identified by most simulation runs at most dose combinations.

Table B.1: Evaluation of f_{12} at all doses, for full model of Scenario 1.

DrugA	DrugB	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	1.25	0.400	0.405	0.005	0.012	0.014	0.93	0	7	93
5.0	1.25	0.400	0.412	0.012	0.031	0.009	0.97	0	1	99
7.5	1.25	0.400	0.418	0.018	0.046	0.010	0.97	0	1	99
10.0	1.25	0.400	0.423	0.023	0.058	0.012	0.96	0	2	98
15.0	1.25	0.400	0.432	0.032	0.080	0.018	0.96	0	6	94
20.0	1.25	0.400	0.440	0.040	0.099	0.030	0.95	0	22	78
2.5	2.50	0.400	0.399	-0.001	-0.004	0.010	0.92	0	1	99
5.0	2.50	0.400	0.405	0.005	0.014	0.007	0.92	0	0	100
7.5	2.50	0.400	0.411	0.011	0.027	0.008	0.92	0	0	100
10.0	2.50	0.400	0.416	0.016	0.039	0.009	0.95	0	0	100
15.0	2.50	0.400	0.424	0.024	0.059	0.013	0.98	0	0	100
20.0	2.50	0.400	0.431	0.031	0.077	0.022	0.96	0	13	87
2.5	3.75	0.400	0.398	-0.002	-0.006	0.011	0.92	0	2	98
5.0	3.75	0.400	0.404	0.004	0.010	0.009	0.91	0	0	100
7.5	3.75	0.400	0.409	0.009	0.023	0.010	0.93	0	0	100
10.0	3.75	0.400	0.413	0.013	0.034	0.011	0.93	0	0	100
15.0	3.75	0.400	0.421	0.021	0.052	0.014	0.97	0	1	99
20.0	3.75	0.400	0.428	0.028	0.069	0.020	0.96	0	10	90
2.5	5.00	0.400	0.399	-0.001	-0.001	0.013	0.94	0	5	95
5.0	5.00	0.400	0.405	0.005	0.014	0.011	0.90	0	1	99
7.5	5.00	0.400	0.410	0.010	0.026	0.012	0.92	0	1	99
10.0	5.00	0.400	0.414	0.014	0.036	0.013	0.92	0	1	99
15.0	5.00	0.400	0.421	0.021	0.053	0.015	0.96	0	1	99
20.0	5.00	0.400	0.428	0.028	0.069	0.020	0.96	0	8	92
2.5	7.50	0.400	0.408	0.008	0.020	0.018	0.94	0	14	86
5.0	7.50	0.400	0.413	0.013	0.033	0.015	0.94	0	3	97
7.5	7.50	0.400	0.417	0.017	0.044	0.015	0.95	0	1	99
10.0	7.50	0.400	0.421	0.021	0.053	0.016	0.94	0	1	99
15.0	7.50	0.400	0.427	0.027	0.069	0.017	0.96	0	2	98
20.0	7.50	0.400	0.433	0.033	0.082	0.021	0.98	0	10	90
2.5	10.00	0.400	0.420	0.020	0.050	0.029	0.93	0	32	68
5.0	10.00	0.400	0.425	0.025	0.062	0.024	0.95	0	21	79
7.5	10.00	0.400	0.429	0.029	0.072	0.023	0.96	0	12	88
10.0	10.00	0.400	0.432	0.032	0.080	0.023	0.96	0	12	88
15.0	10.00	0.400	0.438	0.038	0.094	0.023	0.96	0	12	88
20.0	10.00	0.400	0.443	0.043	0.107	0.028	0.96	0	21	79

Table B.2 shows the detailed results from the evaluation of f_{13}, based on the full model. The function's constant true value of zero indicates additivity, and this is correctly identified by most simulation runs at most dose combinations.

Table B.2: Evaluation of f_{13} at all doses, for full model of Scenario 1.

DrugA	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	0.625	0.000	-0.013	-0.013	.	0.012	0.93	6	93	1
5.0	0.625	0.000	-0.005	-0.005	.	0.008	0.94	4	94	2
7.5	0.625	0.000	0.000	0.000	.	0.009	0.94	4	94	2
10.0	0.625	0.000	0.004	0.004	.	0.012	0.96	2	96	2
15.0	0.625	0.000	0.011	0.011	.	0.018	0.94	3	94	3
20.0	0.625	0.000	0.016	0.016	.	0.029	0.95	2	95	3
2.5	1.250	0.000	-0.009	-0.009	.	0.007	0.93	6	93	1
5.0	1.250	0.000	-0.003	-0.003	.	0.004	0.95	4	95	1
7.5	1.250	0.000	0.001	0.001	.	0.005	0.95	3	95	2
10.0	1.250	0.000	0.004	0.004	.	0.006	0.95	3	95	2
15.0	1.250	0.000	0.009	0.009	.	0.010	0.96	2	96	2
20.0	1.250	0.000	0.013	0.013	.	0.018	0.99	1	99	0
2.5	1.875	0.000	-0.005	-0.005	.	0.008	0.93	3	93	4
5.0	1.875	0.000	-0.001	-0.001	.	0.005	0.95	1	95	4
7.5	1.875	0.000	0.003	0.003	.	0.005	0.92	5	92	3
10.0	1.875	0.000	0.005	0.005	.	0.006	0.95	4	95	1
15.0	1.875	0.000	0.009	0.009	.	0.009	0.96	3	96	1
20.0	1.875	0.000	0.012	0.012	.	0.014	0.97	2	97	1
2.5	2.500	0.000	-0.002	-0.002	.	0.009	0.92	4	92	4
5.0	2.500	0.000	0.002	0.002	.	0.006	0.94	2	94	4
7.5	2.500	0.000	0.005	0.005	.	0.006	0.93	5	93	2
10.0	2.500	0.000	0.007	0.007	.	0.006	0.95	4	95	1
15.0	2.500	0.000	0.010	0.010	.	0.008	0.96	2	96	2
20.0	2.500	0.000	0.011	0.011	.	0.012	0.95	4	95	1
2.5	3.750	0.000	0.006	0.006	.	0.015	0.93	4	93	3
5.0	3.750	0.000	0.009	0.009	.	0.009	0.94	4	94	2
7.5	3.750	0.000	0.010	0.010	.	0.008	0.97	3	97	0
10.0	3.750	0.000	0.011	0.011	.	0.008	0.95	4	95	1
15.0	3.750	0.000	0.012	0.012	.	0.009	0.96	4	96	0
20.0	3.750	0.000	0.012	0.012	.	0.012	0.94	5	94	1
2.5	5.000	0.000	0.015	0.015	.	0.024	0.92	5	92	3
5.0	5.000	0.000	0.016	0.016	.	0.015	0.94	5	94	1
7.5	5.000	0.000	0.016	0.016	.	0.012	0.97	3	97	0
10.0	5.000	0.000	0.016	0.016	.	0.012	0.96	3	96	1
15.0	5.000	0.000	0.016	0.016	.	0.012	0.95	4	95	1
20.0	5.000	0.000	0.015	0.015	.	0.015	0.96	4	96	0

Table B.3 shows the detailed results from the evaluation of f_{23}, based on the full model. The function's constant true value of zero indicates additivity, and this is correctly identified by most simulation runs at most dose combinations.

Table B.3: Evaluation of f_{23} at all doses, for full model of Scenario 1.

DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
1.25	0.625	0.000	0.007	0.007	.	0.016	0.97	1	97	2
2.50	0.625	0.000	0.013	0.013	.	0.013	0.98	1	98	1
3.75	0.625	0.000	0.015	0.015	.	0.012	0.99	0	99	1
5.00	0.625	0.000	0.016	0.016	.	0.012	0.98	0	98	2
7.50	0.625	0.000	0.012	0.012	.	0.015	0.98	1	98	1
10.00	0.625	0.000	0.004	0.004	.	0.025	0.97	3	97	0
1.25	1.250	0.000	0.004	0.004	.	0.010	0.97	2	97	1
2.50	1.250	0.000	0.010	0.010	.	0.008	0.97	1	97	2
3.75	1.250	0.000	0.013	0.013	.	0.008	0.99	0	99	1
5.00	1.250	0.000	0.013	0.013	.	0.008	0.98	0	98	2
7.50	1.250	0.000	0.010	0.010	.	0.010	0.99	1	99	0
10.00	1.250	0.000	0.002	0.002	.	0.017	0.98	2	98	0
1.25	1.875	0.000	0.003	0.003	.	0.010	0.95	4	95	1
2.50	1.875	0.000	0.009	0.009	.	0.008	0.96	2	96	2
3.75	1.875	0.000	0.012	0.012	.	0.008	0.96	1	96	3
5.00	1.875	0.000	0.012	0.012	.	0.008	0.98	0	98	2
7.50	1.875	0.000	0.009	0.009	.	0.009	0.97	2	97	1
10.00	1.875	0.000	0.002	0.002	.	0.015	0.98	2	98	0
1.25	2.500	0.000	0.002	0.002	.	0.010	0.94	4	94	2
2.50	2.500	0.000	0.008	0.008	.	0.008	0.95	3	95	2
3.75	2.500	0.000	0.011	0.011	.	0.008	0.96	1	96	3
5.00	2.500	0.000	0.012	0.012	.	0.008	0.96	2	96	2
7.50	2.500	0.000	0.009	0.009	.	0.009	0.97	2	97	1
10.00	2.500	0.000	0.002	0.002	.	0.014	1.00	0	100	0
1.25	3.750	0.000	0.001	0.001	.	0.014	0.95	4	95	1
2.50	3.750	0.000	0.008	0.008	.	0.009	0.97	2	97	1
3.75	3.750	0.000	0.011	0.011	.	0.009	0.95	4	95	1
5.00	3.750	0.000	0.011	0.011	.	0.008	0.94	5	94	1
7.50	3.750	0.000	0.009	0.009	.	0.009	0.96	4	96	0
10.00	3.750	0.000	0.002	0.002	.	0.012	0.99	1	99	0
1.25	5.000	0.000	0.000	0.000	.	0.022	0.95	4	95	1
2.50	5.000	0.000	0.007	0.007	.	0.015	0.96	3	96	1
3.75	5.000	0.000	0.010	0.010	.	0.013	0.94	4	94	2
5.00	5.000	0.000	0.011	0.011	.	0.011	0.94	4	94	2
7.50	5.000	0.000	0.009	0.009	.	0.011	0.94	5	94	1
10.00	5.000	0.000	0.002	0.002	.	0.013	0.97	2	97	1

Table B.4 shows the detailed results from the evaluation of f_{123}, based on the full model. The function always has a positive true value, indicating antagonism, and this is correctly identified by most simulation runs at most dose combinations.

Table B.4: Evaluation of f_{123} at all doses, for full model of Scenario 1.

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	1.25	0.625	0.183	0.161	-0.022	-0.121	0.050	0.93	0	88	12
5.0	1.25	0.625	0.231	0.205	-0.026	-0.114	0.032	0.96	0	78	22
7.5	1.25	0.625	0.265	0.236	-0.029	-0.109	0.027	0.98	0	72	28
10.0	1.25	0.625	0.293	0.262	-0.031	-0.105	0.025	0.99	0	66	34
15.0	1.25	0.625	0.336	0.303	-0.033	-0.099	0.026	0.99	0	64	36
20.0	1.25	0.625	0.370	0.336	-0.035	-0.094	0.039	0.98	0	70	30
2.5	2.50	0.625	0.231	0.217	-0.014	-0.060	0.038	0.94	0	73	27
5.0	2.50	0.625	0.292	0.274	-0.018	-0.061	0.024	0.92	0	50	50
7.5	2.50	0.625	0.335	0.314	-0.020	-0.061	0.023	0.92	0	41	59
10.0	2.50	0.625	0.369	0.347	-0.022	-0.060	0.022	0.93	0	34	66
15.0	2.50	0.625	0.424	0.399	-0.024	-0.058	0.026	0.95	0	34	66
20.0	2.50	0.625	0.467	0.441	-0.026	-0.055	0.040	0.98	0	42	58
2.5	3.75	0.625	0.265	0.256	-0.009	-0.034	0.036	0.91	0	65	35
5.0	3.75	0.625	0.335	0.322	-0.013	-0.039	0.025	0.91	0	41	59
7.5	3.75	0.625	0.384	0.368	-0.015	-0.040	0.025	0.91	0	31	69
10.0	3.75	0.625	0.423	0.406	-0.017	-0.040	0.026	0.91	0	23	77
15.0	3.75	0.625	0.485	0.466	-0.019	-0.039	0.029	0.94	0	18	82
20.0	3.75	0.625	0.535	0.515	-0.020	-0.038	0.043	0.98	0	29	71
2.5	5.00	0.625	0.292	0.286	-0.006	-0.020	0.034	0.93	0	57	43
5.0	5.00	0.625	0.369	0.359	-0.010	-0.026	0.026	0.93	0	35	65
7.5	5.00	0.625	0.423	0.411	-0.012	-0.028	0.027	0.91	0	22	78
10.0	5.00	0.625	0.466	0.452	-0.014	-0.029	0.028	0.91	0	17	83
15.0	5.00	0.625	0.534	0.519	-0.016	-0.029	0.031	0.92	0	12	88
20.0	5.00	0.625	0.589	0.573	-0.017	-0.028	0.044	0.97	0	25	75
2.5	7.50	0.625	0.335	0.334	-0.002	-0.005	0.032	0.97	0	54	46
5.0	7.50	0.625	0.423	0.418	-0.005	-0.013	0.028	0.95	0	27	73
7.5	7.50	0.625	0.485	0.477	-0.008	-0.016	0.030	0.93	0	20	80
10.0	7.50	0.625	0.534	0.525	-0.009	-0.017	0.031	0.93	0	14	86
15.0	7.50	0.625	0.613	0.602	-0.011	-0.017	0.033	0.97	0	12	88
20.0	7.50	0.625	0.675	0.664	-0.011	-0.017	0.044	0.98	0	15	85
2.5	10.00	0.625	0.370	0.371	0.001	0.003	0.041	0.98	0	62	38
5.0	10.00	0.625	0.466	0.464	-0.002	-0.005	0.039	0.98	0	43	57
7.5	10.00	0.625	0.534	0.530	-0.005	-0.009	0.042	0.97	0	29	71
10.0	10.00	0.625	0.589	0.583	-0.006	-0.010	0.043	0.97	0	24	76
15.0	10.00	0.625	0.675	0.668	-0.007	-0.011	0.043	0.99	0	16	84
20.0	10.00	0.625	0.744	0.736	-0.008	-0.011	0.051	0.99	0	16	84
2.5	1.25	1.250	0.231	0.222	-0.008	-0.035	0.030	0.93	0	78	22
5.0	1.25	1.250	0.291	0.279	-0.012	-0.043	0.018	0.98	0	52	48
7.5	1.25	1.250	0.334	0.319	-0.015	-0.045	0.016	0.98	0	42	58
10.0	1.25	1.250	0.369	0.352	-0.017	-0.046	0.015	0.98	0	31	69
15.0	1.25	1.250	0.423	0.404	-0.019	-0.045	0.017	0.98	0	24	76
20.0	1.25	1.250	0.467	0.446	-0.021	-0.044	0.030	0.98	0	37	63
2.5	2.50	1.250	0.291	0.291	0.000	0.000	0.022	0.94	0	47	53
5.0	2.50	1.250	0.368	0.364	-0.004	-0.011	0.012	0.94	0	6	94
7.5	2.50	1.250	0.422	0.415	-0.007	-0.016	0.012	0.92	0	4	96
10.0	2.50	1.250	0.465	0.457	-0.008	-0.018	0.012	0.94	0	3	97
15.0	2.50	1.250	0.534	0.523	-0.010	-0.020	0.015	0.97	0	3	97
20.0	2.50	1.250	0.589	0.577	-0.012	-0.020	0.028	0.98	0	13	87
2.5	3.75	1.250	0.334	0.339	0.005	0.014	0.022	0.97	0	35	65
5.0	3.75	1.250	0.422	0.423	0.001	0.002	0.014	0.93	0	4	96
7.5	3.75	1.250	0.484	0.482	-0.002	-0.003	0.014	0.92	0	4	96
10.0	3.75	1.250	0.533	0.530	-0.003	-0.006	0.015	0.91	0	1	99
15.0	3.75	1.250	0.611	0.606	-0.005	-0.009	0.017	0.94	0	2	98
20.0	3.75	1.250	0.674	0.668	-0.006	-0.010	0.030	0.95	0	6	94
2.5	5.00	1.250	0.368	0.376	0.008	0.022	0.022	0.96	0	30	70
5.0	5.00	1.250	0.465	0.469	0.004	0.009	0.015	0.92	0	3	97
7.5	5.00	1.250	0.533	0.535	0.002	0.003	0.016	0.89	0	1	99

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Table B.4 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
10.0	5.00	1.250	0.587	0.587	0.000	0.000	0.016	0.89	0	0	100
15.0	5.00	1.250	0.674	0.672	-0.002	-0.003	0.018	0.92	0	0	100
20.0	5.00	1.250	0.742	0.740	-0.003	-0.004	0.029	0.96	0	4	96
2.5	7.50	1.250	0.423	0.435	0.012	0.029	0.022	0.98	0	26	74
5.0	7.50	1.250	0.533	0.541	0.008	0.015	0.017	0.95	0	0	100
7.5	7.50	1.250	0.611	0.617	0.006	0.010	0.019	0.92	0	0	100
10.0	7.50	1.250	0.673	0.678	0.005	0.007	0.019	0.92	0	0	100
15.0	7.50	1.250	0.772	0.775	0.003	0.004	0.019	0.94	0	0	100
20.0	7.50	1.250	0.851	0.853	0.002	0.003	0.027	0.97	0	0	100
2.5	10.00	1.250	0.466	0.481	0.015	0.032	0.032	1.00	0	34	66
5.0	10.00	1.250	0.588	0.599	0.011	0.019	0.029	0.97	0	7	93
7.5	10.00	1.250	0.673	0.682	0.009	0.013	0.031	0.96	0	4	96
10.0	10.00	1.250	0.742	0.749	0.008	0.010	0.031	0.96	0	0	100
15.0	10.00	1.250	0.851	0.857	0.006	0.007	0.028	0.97	0	0	100
20.0	10.00	1.250	0.937	0.943	0.006	0.006	0.033	0.98	0	0	100
2.5	1.25	1.875	0.264	0.263	-0.001	-0.004	0.025	0.98	0	67	33
5.0	1.25	1.875	0.334	0.328	-0.005	-0.016	0.015	0.97	0	32	68
7.5	1.25	1.875	0.383	0.375	-0.008	-0.021	0.015	0.96	0	18	82
10.0	1.25	1.875	0.422	0.412	-0.010	-0.023	0.015	0.98	0	8	92
15.0	1.25	1.875	0.485	0.472	-0.012	-0.025	0.017	0.97	0	10	90
20.0	1.25	1.875	0.534	0.521	-0.014	-0.025	0.029	0.96	0	25	75
2.5	2.50	1.875	0.334	0.341	0.007	0.021	0.018	0.97	0	32	68
5.0	2.50	1.875	0.421	0.424	0.003	0.007	0.010	0.94	0	2	98
7.5	2.50	1.875	0.483	0.484	0.000	0.001	0.010	0.93	0	1	99
10.0	2.50	1.875	0.533	0.531	-0.001	-0.002	0.010	0.96	0	0	100
15.0	2.50	1.875	0.611	0.608	-0.003	-0.006	0.013	0.95	0	0	100
20.0	2.50	1.875	0.674	0.669	-0.005	-0.007	0.025	0.98	0	5	95
2.5	3.75	1.875	0.383	0.394	0.012	0.031	0.019	0.97	0	22	78
5.0	3.75	1.875	0.483	0.491	0.008	0.016	0.011	0.94	0	2	98
7.5	3.75	1.875	0.554	0.559	0.005	0.009	0.012	0.93	0	0	100
10.0	3.75	1.875	0.610	0.614	0.004	0.006	0.012	0.95	0	0	100
15.0	3.75	1.875	0.700	0.702	0.002	0.002	0.014	0.94	0	0	100
20.0	3.75	1.875	0.772	0.772	0.001	0.001	0.025	0.97	0	1	99
2.5	5.00	1.875	0.422	0.437	0.015	0.035	0.019	0.96	0	14	86
5.0	5.00	1.875	0.532	0.543	0.011	0.020	0.013	0.95	0	0	100
7.5	5.00	1.875	0.610	0.619	0.009	0.014	0.013	0.92	0	0	100
10.0	5.00	1.875	0.672	0.679	0.007	0.010	0.013	0.91	0	0	100
15.0	5.00	1.875	0.771	0.776	0.005	0.007	0.014	0.93	0	0	100
20.0	5.00	1.875	0.850	0.854	0.004	0.005	0.024	0.96	0	0	100
2.5	7.50	1.875	0.484	0.503	0.019	0.039	0.021	0.98	0	9	91
5.0	7.50	1.875	0.611	0.626	0.015	0.025	0.015	0.95	0	0	100
7.5	7.50	1.875	0.700	0.713	0.013	0.018	0.016	0.94	0	0	100
10.0	7.50	1.875	0.771	0.782	0.011	0.015	0.015	0.93	0	0	100
15.0	7.50	1.875	0.884	0.894	0.010	0.011	0.015	0.94	0	0	100
20.0	7.50	1.875	0.974	0.983	0.009	0.009	0.022	0.97	0	0	100
2.5	10.00	1.875	0.534	0.555	0.022	0.041	0.032	0.99	0	25	75
5.0	10.00	1.875	0.673	0.691	0.018	0.027	0.028	0.97	0	3	97
7.5	10.00	1.875	0.771	0.787	0.016	0.020	0.028	0.95	0	0	100
10.0	10.00	1.875	0.849	0.864	0.014	0.017	0.028	0.95	0	0	100
15.0	10.00	1.875	0.974	0.987	0.013	0.013	0.025	0.95	0	0	100
20.0	10.00	1.875	1.073	1.085	0.012	0.012	0.030	0.96	0	0	100
2.5	1.25	2.500	0.291	0.293	0.003	0.009	0.023	0.97	0	57	43
5.0	1.25	2.500	0.367	0.366	-0.002	-0.004	0.015	0.95	0	21	79
7.5	1.25	2.500	0.422	0.417	-0.004	-0.010	0.015	0.95	0	8	92
10.0	1.25	2.500	0.465	0.459	-0.006	-0.013	0.016	0.97	0	8	92
15.0	1.25	2.500	0.533	0.525	-0.008	-0.016	0.018	0.97	0	6	94
20.0	1.25	2.500	0.588	0.579	-0.010	-0.016	0.030	0.96	0	15	85
2.5	2.50	2.500	0.367	0.378	0.011	0.029	0.016	0.98	0	19	81

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Table B.4 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
5.0	2.50	2.500	0.464	0.470	0.007	0.014	0.009	0.96	0	0	100
7.5	2.50	2.500	0.532	0.536	0.004	0.008	0.009	0.94	0	0	100
10.0	2.50	2.500	0.586	0.589	0.002	0.004	0.010	0.95	0	0	100
15.0	2.50	2.500	0.673	0.673	0.000	0.000	0.012	0.96	0	0	100
20.0	2.50	2.500	0.742	0.741	-0.001	-0.001	0.023	0.98	0	1	99
2.5	3.75	2.500	0.421	0.437	0.015	0.037	0.018	0.97	0	11	89
5.0	3.75	2.500	0.532	0.543	0.011	0.021	0.010	0.97	0	0	100
7.5	3.75	2.500	0.610	0.619	0.009	0.015	0.011	0.94	0	0	100
10.0	3.75	2.500	0.672	0.679	0.007	0.011	0.011	0.92	0	0	100
15.0	3.75	2.500	0.771	0.776	0.005	0.007	0.012	0.94	0	0	100
20.0	3.75	2.500	0.850	0.854	0.004	0.005	0.023	0.97	0	0	100
2.5	5.00	2.500	0.464	0.483	0.019	0.040	0.019	0.96	0	8	92
5.0	5.00	2.500	0.586	0.601	0.014	0.025	0.011	0.98	0	0	100
7.5	5.00	2.500	0.672	0.684	0.012	0.018	0.012	0.94	0	0	100
10.0	5.00	2.500	0.740	0.751	0.011	0.014	0.011	0.93	0	0	100
15.0	5.00	2.500	0.849	0.858	0.009	0.010	0.012	0.95	0	0	100
20.0	5.00	2.500	0.936	0.944	0.008	0.008	0.022	0.96	0	0	100
2.5	7.50	2.500	0.533	0.556	0.023	0.043	0.021	0.99	0	3	97
5.0	7.50	2.500	0.672	0.691	0.019	0.028	0.014	0.97	0	0	100
7.5	7.50	2.500	0.770	0.787	0.016	0.021	0.014	0.95	0	0	100
10.0	7.50	2.500	0.849	0.864	0.015	0.018	0.014	0.95	0	0	100
15.0	7.50	2.500	0.973	0.986	0.013	0.014	0.013	0.96	0	0	100
20.0	7.50	2.500	1.072	1.085	0.013	0.012	0.020	0.96	0	0	100
2.5	10.00	2.500	0.587	0.613	0.026	0.043	0.033	0.99	0	15	85
5.0	10.00	2.500	0.741	0.762	0.021	0.029	0.027	0.97	0	1	99
7.5	10.00	2.500	0.849	0.868	0.019	0.023	0.027	0.95	0	0	100
10.0	10.00	2.500	0.935	0.953	0.018	0.019	0.027	0.95	0	0	100
15.0	10.00	2.500	1.072	1.088	0.016	0.015	0.024	0.95	0	0	100
20.0	10.00	2.500	1.181	1.197	0.016	0.014	0.030	0.97	0	0	100
2.5	1.25	3.750	0.333	0.338	0.005	0.015	0.026	0.96	0	49	51
5.0	1.25	3.750	0.421	0.421	0.001	0.001	0.019	0.96	0	17	83
7.5	1.25	3.750	0.483	0.481	-0.002	-0.004	0.019	0.95	0	6	94
10.0	1.25	3.750	0.532	0.528	-0.004	-0.007	0.020	0.95	0	3	97
15.0	1.25	3.750	0.611	0.605	-0.006	-0.010	0.021	0.97	0	3	97
20.0	1.25	3.750	0.674	0.666	-0.007	-0.011	0.033	0.98	0	8	92
2.5	2.50	3.750	0.421	0.434	0.013	0.031	0.018	0.98	0	16	84
5.0	2.50	3.750	0.531	0.540	0.009	0.016	0.010	0.98	0	0	100
7.5	2.50	3.750	0.609	0.615	0.006	0.010	0.010	0.99	0	0	100
10.0	2.50	3.750	0.671	0.676	0.005	0.007	0.010	0.99	0	0	100
15.0	2.50	3.750	0.770	0.773	0.002	0.003	0.011	0.97	0	0	100
20.0	2.50	3.750	0.849	0.851	0.001	0.002	0.022	0.98	0	0	100
2.5	3.75	3.750	0.483	0.500	0.018	0.036	0.019	0.96	0	5	95
5.0	3.75	3.750	0.609	0.622	0.013	0.022	0.011	0.99	0	0	100
7.5	3.75	3.750	0.698	0.709	0.011	0.016	0.010	0.99	0	0	100
10.0	3.75	3.750	0.769	0.779	0.009	0.012	0.010	0.97	0	0	100
15.0	3.75	3.750	0.882	0.890	0.007	0.008	0.010	0.96	0	0	100
20.0	3.75	3.750	0.973	0.979	0.006	0.007	0.021	0.96	0	0	100
2.5	5.00	3.750	0.532	0.553	0.021	0.039	0.020	0.96	0	4	96
5.0	5.00	3.750	0.671	0.688	0.017	0.025	0.011	0.99	0	0	100
7.5	5.00	3.750	0.769	0.783	0.014	0.018	0.011	0.98	0	0	100
10.0	5.00	3.750	0.848	0.860	0.013	0.015	0.010	0.97	0	0	100
15.0	5.00	3.750	0.972	0.983	0.011	0.011	0.010	0.96	0	0	100
20.0	5.00	3.750	1.071	1.081	0.010	0.009	0.020	0.96	0	0	100
2.5	7.50	3.750	0.610	0.635	0.025	0.041	0.023	0.99	0	1	99
5.0	7.50	3.750	0.770	0.791	0.021	0.027	0.014	0.98	0	0	100
7.5	7.50	3.750	0.882	0.900	0.018	0.021	0.014	0.95	0	0	100
10.0	7.50	3.750	0.972	0.989	0.017	0.018	0.013	0.94	0	0	100
15.0	7.50	3.750	1.114	1.129	0.015	0.014	0.013	0.97	0	0	100

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Table B.4 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
20.0	7.50	3.750	1.228	1.242	0.015	0.012	0.022	0.95	0	0	100
2.5	10.00	3.750	0.673	0.700	0.028	0.041	0.037	1.00	0	8	92
5.0	10.00	3.750	0.848	0.872	0.024	0.028	0.029	0.96	0	0	100
7.5	10.00	3.750	0.972	0.993	0.021	0.022	0.028	0.95	0	0	100
10.0	10.00	3.750	1.071	1.091	0.020	0.019	0.028	0.95	0	0	100
15.0	10.00	3.750	1.227	1.246	0.019	0.015	0.028	0.96	0	0	100
20.0	10.00	3.750	1.352	1.370	0.018	0.013	0.037	0.95	0	0	100
2.5	1.25	5.000	0.367	0.370	0.003	0.008	0.039	0.96	0	53	47
5.0	1.25	5.000	0.463	0.462	-0.001	-0.003	0.032	0.95	0	27	73
7.5	1.25	5.000	0.531	0.528	-0.004	-0.007	0.032	0.95	0	13	87
10.0	1.25	5.000	0.586	0.580	-0.006	-0.010	0.032	0.94	0	9	91
15.0	1.25	5.000	0.672	0.665	-0.008	-0.012	0.033	0.95	0	6	94
20.0	1.25	5.000	0.741	0.732	-0.009	-0.012	0.044	0.97	0	8	92
2.5	2.50	5.000	0.463	0.474	0.011	0.024	0.029	0.99	0	31	69
5.0	2.50	5.000	0.585	0.592	0.007	0.012	0.020	0.99	0	2	98
7.5	2.50	5.000	0.671	0.675	0.004	0.006	0.020	0.99	0	1	99
10.0	2.50	5.000	0.739	0.742	0.003	0.004	0.019	0.97	0	0	100
15.0	2.50	5.000	0.848	0.849	0.001	0.001	0.020	0.97	0	0	100
20.0	2.50	5.000	0.935	0.934	0.000	-0.001	0.030	0.96	0	0	100
2.5	3.75	5.000	0.531	0.547	0.016	0.030	0.030	0.99	0	17	83
5.0	3.75	5.000	0.671	0.682	0.011	0.017	0.020	1.00	0	0	100
7.5	3.75	5.000	0.769	0.778	0.009	0.012	0.018	1.00	0	0	100
10.0	3.75	5.000	0.847	0.854	0.007	0.009	0.018	1.00	0	0	100
15.0	3.75	5.000	0.971	0.977	0.006	0.006	0.018	0.96	0	0	100
20.0	3.75	5.000	1.071	1.075	0.005	0.004	0.028	0.96	0	0	100
2.5	5.00	5.000	0.586	0.605	0.019	0.032	0.030	0.99	0	11	89
5.0	5.00	5.000	0.739	0.754	0.015	0.020	0.020	0.98	0	0	100
7.5	5.00	5.000	0.847	0.859	0.012	0.015	0.018	0.98	0	0	100
10.0	5.00	5.000	0.933	0.944	0.011	0.012	0.017	0.98	0	0	100
15.0	5.00	5.000	1.070	1.079	0.009	0.008	0.018	0.96	0	0	100
20.0	5.00	5.000	1.179	1.187	0.008	0.007	0.029	0.96	0	0	100
2.5	7.50	5.000	0.672	0.695	0.023	0.034	0.034	0.99	0	5	95
5.0	7.50	5.000	0.848	0.866	0.019	0.022	0.023	0.98	0	0	100
7.5	7.50	5.000	0.971	0.988	0.017	0.017	0.022	0.98	0	0	100
10.0	7.50	5.000	1.070	1.085	0.015	0.014	0.022	0.97	0	0	100
15.0	7.50	5.000	1.226	1.240	0.014	0.011	0.023	0.94	0	0	100
20.0	7.50	5.000	1.351	1.364	0.013	0.010	0.035	0.95	0	0	100
2.5	10.00	5.000	0.741	0.767	0.026	0.035	0.049	0.98	0	10	90
5.0	10.00	5.000	0.934	0.956	0.022	0.023	0.038	0.98	0	0	100
7.5	10.00	5.000	1.070	1.090	0.019	0.018	0.038	0.96	0	0	100
10.0	10.00	5.000	1.179	1.197	0.018	0.015	0.039	0.96	0	0	100
15.0	10.00	5.000	1.351	1.368	0.017	0.012	0.042	0.94	0	0	100
20.0	10.00	5.000	1.489	1.505	0.016	0.011	0.056	0.92	0	0	100

B.1.2 Final Model

Table B.5 shows the detailed results from the evaluation of f_{12}, based on the final model. The function has a constant true value of 0.4, indicating antagonism, and this is correctly identified by most simulation runs at most dose combinations.

Table B.5: Evaluation of f_{12} at all doses, for final model of Scenario 1.

DrugA	DrugB	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	1.25	0.400	0.408	0.008	0.019	0.012	0.87	0	3	97
5.0	1.25	0.400	0.412	0.012	0.030	0.008	0.88	0	0	100
7.5	1.25	0.400	0.415	0.015	0.037	0.009	0.89	0	0	100
10.0	1.25	0.400	0.417	0.017	0.042	0.010	0.90	0	1	99
15.0	1.25	0.400	0.419	0.019	0.049	0.015	0.88	0	2	98
20.0	1.25	0.400	0.421	0.021	0.052	0.024	0.88	0	7	93
2.5	2.50	0.400	0.399	-0.001	-0.002	0.009	0.87	0	0	100
5.0	2.50	0.400	0.405	0.005	0.012	0.007	0.89	0	0	100
7.5	2.50	0.400	0.408	0.008	0.021	0.007	0.87	0	0	100
10.0	2.50	0.400	0.411	0.011	0.027	0.008	0.88	0	0	100
15.0	2.50	0.400	0.415	0.015	0.037	0.012	0.90	0	0	100
20.0	2.50	0.400	0.417	0.017	0.042	0.018	0.87	0	3	97
2.5	3.75	0.400	0.394	-0.006	-0.015	0.010	0.88	0	1	99
5.0	3.75	0.400	0.400	0.000	0.001	0.007	0.87	0	0	100
7.5	3.75	0.400	0.405	0.005	0.012	0.008	0.88	0	0	100
10.0	3.75	0.400	0.408	0.008	0.020	0.009	0.89	0	0	100
15.0	3.75	0.400	0.413	0.013	0.031	0.012	0.90	0	0	100
20.0	3.75	0.400	0.416	0.016	0.039	0.016	0.87	0	2	98
2.5	5.00	0.400	0.391	-0.009	-0.024	0.011	0.84	0	2	98
5.0	5.00	0.400	0.398	-0.002	-0.006	0.009	0.84	0	0	100
7.5	5.00	0.400	0.403	0.003	0.007	0.009	0.86	0	0	100
10.0	5.00	0.400	0.406	0.006	0.016	0.010	0.88	0	0	100
15.0	5.00	0.400	0.412	0.012	0.029	0.012	0.89	0	0	100
20.0	5.00	0.400	0.416	0.016	0.039	0.016	0.87	0	3	97
2.5	7.50	0.400	0.387	-0.013	-0.032	0.016	0.85	0	4	96
5.0	7.50	0.400	0.396	-0.004	-0.011	0.012	0.85	0	1	99
7.5	7.50	0.400	0.401	0.001	0.004	0.012	0.87	0	0	100
10.0	7.50	0.400	0.406	0.006	0.015	0.012	0.89	0	0	100
15.0	7.50	0.400	0.413	0.013	0.032	0.013	0.91	0	0	100
20.0	7.50	0.400	0.418	0.018	0.044	0.016	0.90	0	2	98
2.5	10.00	0.400	0.386	-0.014	-0.034	0.026	0.82	0	11	89
5.0	10.00	0.400	0.396	-0.004	-0.010	0.020	0.85	0	9	91
7.5	10.00	0.400	0.403	0.003	0.007	0.018	0.84	0	6	94
10.0	10.00	0.400	0.408	0.008	0.020	0.017	0.87	0	3	97
15.0	10.00	0.400	0.416	0.016	0.039	0.017	0.92	0	3	97
20.0	10.00	0.400	0.422	0.022	0.054	0.020	0.92	0	4	96

Table B.6 shows the detailed results from the evaluation of f_{13}, based on the final model. The function's constant true value of zero indicates additivity, and this is correctly identified by most simulation runs at most dose combinations.

Table B.7 shows the detailed results from the evaluation of f_{23}, based on the final

Table B.6: Evaluation of f_{13} at all doses, for final model of Scenario 1.

DrugA	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	0.625	0.000	-0.005	-0.005	.	0.009	0.87	10	87	3
5.0	0.625	0.000	-0.005	-0.005	.	0.007	0.89	10	89	1
7.5	0.625	0.000	-0.004	-0.004	.	0.008	0.86	11	86	3
10.0	0.625	0.000	-0.003	-0.003	.	0.009	0.84	12	84	4
15.0	0.625	0.000	0.001	0.001	.	0.014	0.83	11	83	6
20.0	0.625	0.000	0.005	0.005	.	0.020	0.82	11	82	7
2.5	1.250	0.000	-0.004	-0.004	.	0.005	0.91	7	91	2
5.0	1.250	0.000	-0.004	-0.004	.	0.004	0.93	6	93	1
7.5	1.250	0.000	-0.003	-0.003	.	0.005	0.88	9	88	3
10.0	1.250	0.000	-0.002	-0.002	.	0.006	0.88	9	88	3
15.0	1.250	0.000	0.001	0.001	.	0.009	0.87	9	87	4
20.0	1.250	0.000	0.005	0.005	.	0.013	0.87	10	87	3
2.5	1.875	0.000	-0.003	-0.003	.	0.005	0.92	6	92	2
5.0	1.875	0.000	-0.003	-0.003	.	0.004	0.92	6	92	2
7.5	1.875	0.000	-0.002	-0.002	.	0.004	0.88	9	88	3
10.0	1.875	0.000	-0.001	-0.001	.	0.005	0.87	9	87	4
15.0	1.875	0.000	0.002	0.002	.	0.008	0.86	8	86	6
20.0	1.875	0.000	0.006	0.006	.	0.011	0.86	9	86	5
2.5	2.500	0.000	-0.002	-0.002	.	0.006	0.91	7	91	2
5.0	2.500	0.000	-0.002	-0.002	.	0.004	0.93	5	93	2
7.5	2.500	0.000	-0.001	-0.001	.	0.005	0.88	8	88	4
10.0	2.500	0.000	0.001	0.001	.	0.006	0.87	9	87	4
15.0	2.500	0.000	0.004	0.004	.	0.007	0.87	7	87	6
20.0	2.500	0.000	0.007	0.007	.	0.010	0.88	7	88	5
2.5	3.750	0.000	0.002	0.002	.	0.009	0.86	10	86	4
5.0	3.750	0.000	0.002	0.002	.	0.007	0.91	7	91	2
7.5	3.750	0.000	0.003	0.003	.	0.006	0.89	8	89	3
10.0	3.750	0.000	0.004	0.004	.	0.007	0.88	8	88	4
15.0	3.750	0.000	0.007	0.007	.	0.008	0.89	7	89	4
20.0	3.750	0.000	0.011	0.011	.	0.009	0.89	7	89	4
2.5	5.000	0.000	0.006	0.006	.	0.014	0.83	10	83	7
5.0	5.000	0.000	0.006	0.006	.	0.010	0.89	8	89	3
7.5	5.000	0.000	0.007	0.007	.	0.009	0.90	8	90	2
10.0	5.000	0.000	0.008	0.008	.	0.009	0.90	8	90	2
15.0	5.000	0.000	0.011	0.011	.	0.009	0.89	7	89	4
20.0	5.000	0.000	0.014	0.014	.	0.011	0.89	6	89	5

model. The function's constant true value of zero indicates additivity, and this is correctly identified by most simulation runs at most dose combinations.

Table B.7: Evaluation of f_{23} at all doses, for final model of Scenario 1.

DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
1.25	0.625	0.000	0.027	0.027	.	0.014	0.90	5	90	5
2.50	0.625	0.000	0.020	0.020	.	0.010	0.94	4	94	2
3.75	0.625	0.000	0.013	0.013	.	0.008	0.94	3	94	3
5.00	0.625	0.000	0.007	0.007	.	0.008	0.94	3	94	3
7.50	0.625	0.000	-0.006	-0.006	.	0.010	0.96	3	96	1
10.00	0.625	0.000	-0.020	-0.020	.	0.017	0.91	7	91	2
1.25	1.250	0.000	0.022	0.022	.	0.010	0.92	3	92	5
2.50	1.250	0.000	0.017	0.017	.	0.007	0.93	4	93	3
3.75	1.250	0.000	0.012	0.012	.	0.006	0.94	4	94	2
5.00	1.250	0.000	0.007	0.007	.	0.006	0.96	2	96	2
7.50	1.250	0.000	-0.004	-0.004	.	0.007	0.96	3	96	1
10.00	1.250	0.000	-0.016	-0.016	.	0.012	0.93	6	93	1
1.25	1.875	0.000	0.017	0.017	.	0.009	0.91	3	91	6
2.50	1.875	0.000	0.014	0.014	.	0.007	0.91	4	91	5
3.75	1.875	0.000	0.010	0.010	.	0.006	0.94	3	94	3
5.00	1.875	0.000	0.006	0.006	.	0.006	0.95	2	95	3
7.50	1.875	0.000	-0.003	-0.003	.	0.007	0.94	4	94	2
10.00	1.875	0.000	-0.013	-0.013	.	0.011	0.91	7	91	2
1.25	2.500	0.000	0.013	0.013	.	0.010	0.89	5	89	6
2.50	2.500	0.000	0.011	0.011	.	0.007	0.91	4	91	5
3.75	2.500	0.000	0.008	0.008	.	0.006	0.92	4	92	4
5.00	2.500	0.000	0.005	0.005	.	0.006	0.94	3	94	3
7.50	2.500	0.000	-0.003	-0.003	.	0.007	0.93	5	93	2
10.00	2.500	0.000	-0.011	-0.011	.	0.010	0.90	8	90	2
1.25	3.750	0.000	0.004	0.004	.	0.012	0.90	5	90	5
2.50	3.750	0.000	0.005	0.005	.	0.008	0.92	5	92	3
3.75	3.750	0.000	0.004	0.004	.	0.007	0.92	5	92	3
5.00	3.750	0.000	0.002	0.002	.	0.007	0.91	6	91	3
7.50	3.750	0.000	-0.003	-0.003	.	0.007	0.91	6	91	3
10.00	3.750	0.000	-0.010	-0.010	.	0.009	0.90	8	90	2
1.25	5.000	0.000	-0.004	-0.004	.	0.016	0.89	7	89	4
2.50	5.000	0.000	-0.002	-0.002	.	0.011	0.90	7	90	3
3.75	5.000	0.000	-0.001	-0.001	.	0.010	0.89	8	89	3
5.00	5.000	0.000	-0.002	-0.002	.	0.009	0.90	7	90	3
7.50	5.000	0.000	-0.005	-0.005	.	0.009	0.88	9	88	3
10.00	5.000	0.000	-0.009	-0.009	.	0.011	0.88	10	88	2

Table B.8 shows the detailed results from the evaluation of f_{123}, based on the final model. The function always has a positive true value, indicating antagonism, and this is correctly identified by most simulation runs at most dose combinations.

Table B.8: Evaluation of f_{123} at all doses, for final model of Scenario 1.

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	1.25	0.625	0.183	0.118	-0.065	-0.356	0.053	0.74	1	78	21
5.0	1.25	0.625	0.231	0.178	-0.053	-0.230	0.033	0.84	0	65	35
7.5	1.25	0.625	0.265	0.219	-0.046	-0.173	0.026	0.89	0	54	46

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Table B.8 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
10.0	1.25	0.625	0.293	0.252	-0.041	-0.139	0.022	0.91	0	46	54
15.0	1.25	0.625	0.336	0.302	-0.034	-0.101	0.021	0.93	0	37	63
20.0	1.25	0.625	0.370	0.340	-0.030	-0.081	0.027	0.94	0	33	67
2.5	2.50	0.625	0.231	0.187	-0.044	-0.189	0.035	0.81	1	60	39
5.0	2.50	0.625	0.292	0.257	-0.035	-0.118	0.023	0.84	0	42	58
7.5	2.50	0.625	0.335	0.306	-0.029	-0.087	0.019	0.87	0	25	75
10.0	2.50	0.625	0.369	0.344	-0.025	-0.069	0.018	0.92	0	15	85
15.0	2.50	0.625	0.424	0.403	-0.021	-0.049	0.019	0.93	0	10	90
20.0	2.50	0.625	0.467	0.449	-0.018	-0.040	0.026	0.91	0	15	85
2.5	3.75	0.625	0.265	0.238	-0.027	-0.103	0.028	0.82	0	49	51
5.0	3.75	0.625	0.335	0.315	-0.020	-0.060	0.019	0.87	0	21	79
7.5	3.75	0.625	0.384	0.368	-0.016	-0.041	0.018	0.89	0	9	91
10.0	3.75	0.625	0.423	0.410	-0.013	-0.031	0.018	0.90	0	4	96
15.0	3.75	0.625	0.485	0.475	-0.010	-0.021	0.020	0.93	0	5	95
20.0	3.75	0.625	0.535	0.526	-0.009	-0.017	0.028	0.91	0	9	91
2.5	5.00	0.625	0.292	0.279	-0.013	-0.045	0.025	0.86	0	40	60
5.0	5.00	0.625	0.369	0.362	-0.007	-0.020	0.018	0.89	0	12	88
7.5	5.00	0.625	0.423	0.419	-0.004	-0.009	0.017	0.89	0	3	97
10.0	5.00	0.625	0.466	0.464	-0.002	-0.004	0.018	0.90	0	3	97
15.0	5.00	0.625	0.534	0.534	0.000	-0.001	0.021	0.91	0	2	98
20.0	5.00	0.625	0.589	0.589	0.000	-0.001	0.029	0.92	0	5	95
2.5	7.50	0.625	0.335	0.347	0.012	0.035	0.023	0.91	0	28	72
5.0	7.50	0.625	0.423	0.438	0.015	0.036	0.019	0.88	0	4	96
7.5	7.50	0.625	0.485	0.501	0.017	0.034	0.020	0.88	0	3	97
10.0	7.50	0.625	0.534	0.551	0.017	0.032	0.021	0.87	0	3	97
15.0	7.50	0.625	0.613	0.630	0.017	0.028	0.024	0.89	0	2	98
20.0	7.50	0.625	0.675	0.691	0.015	0.023	0.031	0.90	0	3	97
2.5	10.00	0.625	0.370	0.403	0.033	0.090	0.030	0.89	0	25	75
5.0	10.00	0.625	0.466	0.501	0.035	0.075	0.027	0.91	0	8	92
7.5	10.00	0.625	0.534	0.570	0.035	0.066	0.028	0.89	0	4	96
10.0	10.00	0.625	0.589	0.624	0.035	0.059	0.029	0.88	0	3	97
15.0	10.00	0.625	0.675	0.708	0.033	0.049	0.032	0.86	0	2	98
20.0	10.00	0.625	0.744	0.774	0.030	0.041	0.038	0.86	0	2	98
2.5	1.25	1.250	0.231	0.189	-0.041	-0.179	0.030	0.84	0	64	36
5.0	1.25	1.250	0.291	0.259	-0.032	-0.110	0.018	0.89	0	36	64
7.5	1.25	1.250	0.334	0.308	-0.027	-0.080	0.014	0.91	0	25	75
10.0	1.25	1.250	0.369	0.346	-0.023	-0.062	0.013	0.93	0	13	87
15.0	1.25	1.250	0.423	0.405	-0.019	-0.044	0.013	0.92	0	8	92
20.0	1.25	1.250	0.467	0.450	-0.016	-0.035	0.019	0.94	0	10	90
2.5	2.50	1.250	0.291	0.269	-0.023	-0.078	0.020	0.90	0	37	63
5.0	2.50	1.250	0.368	0.351	-0.017	-0.046	0.012	0.92	0	4	96
7.5	2.50	1.250	0.422	0.408	-0.014	-0.032	0.010	0.88	0	3	97
10.0	2.50	1.250	0.465	0.453	-0.012	-0.025	0.010	0.89	0	0	100
15.0	2.50	1.250	0.534	0.524	-0.010	-0.019	0.012	0.94	0	0	100
20.0	2.50	1.250	0.589	0.578	-0.010	-0.017	0.018	0.91	0	1	99
2.5	3.75	1.250	0.334	0.326	-0.008	-0.024	0.017	0.93	0	15	85
5.0	3.75	1.250	0.422	0.417	-0.005	-0.011	0.011	0.91	0	3	97
7.5	3.75	1.250	0.484	0.481	-0.003	-0.006	0.010	0.90	0	1	99
10.0	3.75	1.250	0.533	0.531	-0.002	-0.004	0.010	0.89	0	0	100
15.0	3.75	1.250	0.611	0.609	-0.003	-0.004	0.012	0.94	0	0	100
20.0	3.75	1.250	0.674	0.670	-0.004	-0.006	0.018	0.91	0	1	99
2.5	5.00	1.250	0.368	0.373	0.005	0.013	0.016	0.92	0	7	93
5.0	5.00	1.250	0.465	0.471	0.006	0.014	0.011	0.89	0	1	99
7.5	5.00	1.250	0.533	0.540	0.007	0.012	0.011	0.89	0	0	100
10.0	5.00	1.250	0.587	0.593	0.006	0.011	0.011	0.88	0	0	100
15.0	5.00	1.250	0.674	0.678	0.004	0.006	0.013	0.92	0	0	100
20.0	5.00	1.250	0.742	0.744	0.002	0.002	0.018	0.92	0	0	100
2.5	7.50	1.250	0.423	0.450	0.027	0.064	0.019	0.88	0	4	96

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Table B.8 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
5.0	7.50	1.250	0.533	0.559	0.026	0.048	0.014	0.90	0	0	100
7.5	7.50	1.250	0.611	0.635	0.024	0.039	0.013	0.89	0	0	100
10.0	7.50	1.250	0.673	0.695	0.022	0.033	0.013	0.91	0	0	100
15.0	7.50	1.250	0.772	0.789	0.018	0.023	0.014	0.92	0	0	100
20.0	7.50	1.250	0.851	0.863	0.013	0.015	0.019	0.92	0	0	100
2.5	10.00	1.250	0.466	0.513	0.047	0.101	0.029	0.86	0	7	93
5.0	10.00	1.250	0.588	0.631	0.043	0.074	0.023	0.88	0	0	100
7.5	10.00	1.250	0.673	0.713	0.040	0.059	0.021	0.88	0	0	100
10.0	10.00	1.250	0.742	0.778	0.037	0.049	0.021	0.89	0	0	100
15.0	10.00	1.250	0.851	0.881	0.030	0.035	0.020	0.93	0	0	100
20.0	10.00	1.250	0.937	0.961	0.024	0.025	0.024	0.93	0	0	100
2.5	1.25	1.875	0.264	0.238	-0.026	-0.100	0.022	0.92	0	47	53
5.0	1.25	1.875	0.334	0.315	-0.019	-0.058	0.013	0.94	0	21	79
7.5	1.25	1.875	0.383	0.368	-0.015	-0.040	0.011	0.95	0	4	96
10.0	1.25	1.875	0.422	0.410	-0.013	-0.030	0.011	0.94	0	2	98
15.0	1.25	1.875	0.485	0.475	-0.010	-0.020	0.012	0.93	0	1	99
20.0	1.25	1.875	0.534	0.526	-0.009	-0.017	0.018	0.95	0	3	97
2.5	2.50	1.875	0.334	0.324	-0.010	-0.029	0.015	0.93	0	13	87
5.0	2.50	1.875	0.421	0.415	-0.006	-0.015	0.009	0.92	0	1	99
7.5	2.50	1.875	0.483	0.478	-0.005	-0.010	0.008	0.91	0	0	100
10.0	2.50	1.875	0.533	0.528	-0.004	-0.008	0.008	0.92	0	0	100
15.0	2.50	1.875	0.611	0.607	-0.005	-0.007	0.010	0.94	0	0	100
20.0	2.50	1.875	0.674	0.668	-0.006	-0.009	0.015	0.89	0	0	100
2.5	3.75	1.875	0.383	0.386	0.004	0.010	0.014	0.93	0	4	96
5.0	3.75	1.875	0.483	0.488	0.004	0.009	0.009	0.93	0	1	99
7.5	3.75	1.875	0.554	0.558	0.004	0.007	0.008	0.91	0	0	100
10.0	3.75	1.875	0.610	0.614	0.003	0.005	0.008	0.91	0	0	100
15.0	3.75	1.875	0.700	0.701	0.001	0.001	0.010	0.92	0	0	100
20.0	3.75	1.875	0.772	0.769	-0.003	-0.004	0.015	0.90	0	0	100
2.5	5.00	1.875	0.422	0.437	0.016	0.037	0.015	0.92	0	2	98
5.0	5.00	1.875	0.532	0.546	0.014	0.026	0.010	0.95	0	0	100
7.5	5.00	1.875	0.610	0.622	0.012	0.020	0.009	0.91	0	0	100
10.0	5.00	1.875	0.672	0.683	0.010	0.015	0.009	0.93	0	0	100
15.0	5.00	1.875	0.771	0.777	0.006	0.007	0.010	0.91	0	0	100
20.0	5.00	1.875	0.850	0.851	0.001	0.001	0.015	0.91	0	0	100
2.5	7.50	1.875	0.484	0.520	0.036	0.075	0.019	0.88	0	1	99
5.0	7.50	1.875	0.611	0.642	0.031	0.051	0.013	0.88	0	0	100
7.5	7.50	1.875	0.700	0.727	0.027	0.039	0.012	0.92	0	0	100
10.0	7.50	1.875	0.771	0.794	0.023	0.030	0.011	0.91	0	0	100
15.0	7.50	1.875	0.884	0.900	0.016	0.018	0.011	0.95	0	0	100
20.0	7.50	1.875	0.974	0.983	0.009	0.009	0.015	0.93	0	0	100
2.5	10.00	1.875	0.534	0.588	0.055	0.103	0.030	0.86	0	1	99
5.0	10.00	1.875	0.673	0.720	0.047	0.070	0.022	0.87	0	0	100
7.5	10.00	1.875	0.771	0.812	0.041	0.054	0.019	0.91	0	0	100
10.0	10.00	1.875	0.849	0.885	0.036	0.042	0.018	0.93	0	0	100
15.0	10.00	1.875	0.974	1.000	0.026	0.027	0.017	0.94	0	0	100
20.0	10.00	1.875	1.073	1.090	0.017	0.016	0.021	0.94	0	0	100
2.5	1.25	2.500	0.291	0.275	-0.016	-0.055	0.018	0.92	0	36	64
5.0	1.25	2.500	0.367	0.357	-0.010	-0.028	0.011	0.93	0	8	92
7.5	1.25	2.500	0.422	0.414	-0.007	-0.017	0.010	0.94	0	1	99
10.0	1.25	2.500	0.465	0.459	-0.005	-0.012	0.011	0.94	0	0	100
15.0	1.25	2.500	0.533	0.529	-0.004	-0.007	0.013	0.92	0	0	100
20.0	1.25	2.500	0.588	0.584	-0.004	-0.007	0.019	0.93	0	2	98
2.5	2.50	2.500	0.367	0.367	-0.001	-0.002	0.013	0.94	0	6	94
5.0	2.50	2.500	0.464	0.465	0.001	0.002	0.008	0.94	0	0	100
7.5	2.50	2.500	0.532	0.533	0.001	0.002	0.007	0.93	0	0	100
10.0	2.50	2.500	0.586	0.587	0.001	0.001	0.007	0.92	0	0	100
15.0	2.50	2.500	0.673	0.671	-0.001	-0.002	0.009	0.93	0	0	100

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Table B.8 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
20.0	2.50	2.500	0.742	0.737	-0.004	-0.006	0.014	0.91	0	0	100
2.5	3.75	2.500	0.421	0.433	0.012	0.028	0.013	0.93	0	2	98
5.0	3.75	2.500	0.532	0.542	0.010	0.019	0.008	0.94	0	0	100
7.5	3.75	2.500	0.610	0.618	0.008	0.014	0.008	0.91	0	0	100
10.0	3.75	2.500	0.672	0.678	0.006	0.010	0.008	0.90	0	0	100
15.0	3.75	2.500	0.771	0.772	0.002	0.002	0.009	0.92	0	0	100
20.0	3.75	2.500	0.850	0.847	-0.003	-0.004	0.013	0.92	0	0	100
2.5	5.00	2.500	0.464	0.487	0.023	0.049	0.015	0.88	0	1	99
5.0	5.00	2.500	0.586	0.605	0.019	0.032	0.009	0.94	0	0	100
7.5	5.00	2.500	0.672	0.687	0.015	0.023	0.008	0.95	0	0	100
10.0	5.00	2.500	0.740	0.752	0.012	0.016	0.008	0.90	0	0	100
15.0	5.00	2.500	0.849	0.854	0.005	0.006	0.009	0.90	0	0	100
20.0	5.00	2.500	0.936	0.935	-0.001	-0.001	0.013	0.90	0	0	100
2.5	7.50	2.500	0.533	0.575	0.042	0.079	0.020	0.87	0	0	100
5.0	7.50	2.500	0.672	0.707	0.035	0.052	0.013	0.91	0	0	100
7.5	7.50	2.500	0.770	0.799	0.029	0.037	0.011	0.90	0	0	100
10.0	7.50	2.500	0.849	0.872	0.023	0.027	0.010	0.91	0	0	100
15.0	7.50	2.500	0.973	0.986	0.013	0.014	0.010	0.95	0	0	100
20.0	7.50	2.500	1.072	1.077	0.004	0.004	0.015	0.94	0	0	100
2.5	10.00	2.500	0.587	0.647	0.060	0.102	0.032	0.85	0	1	99
5.0	10.00	2.500	0.741	0.790	0.049	0.067	0.021	0.88	0	0	100
7.5	10.00	2.500	0.849	0.890	0.041	0.049	0.018	0.91	0	0	100
10.0	10.00	2.500	0.935	0.969	0.034	0.037	0.017	0.91	0	0	100
15.0	10.00	2.500	1.072	1.094	0.022	0.020	0.016	0.92	0	0	100
20.0	10.00	2.500	1.181	1.192	0.011	0.009	0.021	0.92	0	0	100
2.5	1.25	3.750	0.333	0.331	-0.002	-0.006	0.018	0.92	0	24	76
5.0	1.25	3.750	0.421	0.422	0.001	0.003	0.013	0.92	0	1	99
7.5	1.25	3.750	0.483	0.485	0.003	0.005	0.013	0.93	0	1	99
10.0	1.25	3.750	0.532	0.535	0.003	0.006	0.014	0.92	0	0	100
15.0	1.25	3.750	0.611	0.613	0.002	0.004	0.017	0.91	0	0	100
20.0	1.25	3.750	0.674	0.674	0.001	0.001	0.022	0.90	0	1	99
2.5	2.50	3.750	0.421	0.432	0.011	0.026	0.014	0.95	0	2	98
5.0	2.50	3.750	0.531	0.541	0.009	0.018	0.009	0.95	0	0	100
7.5	2.50	3.750	0.609	0.617	0.008	0.012	0.009	0.94	0	0	100
10.0	2.50	3.750	0.671	0.677	0.005	0.008	0.009	0.92	0	0	100
15.0	2.50	3.750	0.770	0.771	0.001	0.001	0.010	0.94	0	0	100
20.0	2.50	3.750	0.849	0.845	-0.004	-0.005	0.014	0.94	0	0	100
2.5	3.75	3.750	0.483	0.504	0.022	0.045	0.015	0.92	0	1	99
5.0	3.75	3.750	0.609	0.626	0.017	0.028	0.009	0.96	0	0	100
7.5	3.75	3.750	0.698	0.711	0.013	0.018	0.008	0.94	0	0	100
10.0	3.75	3.750	0.769	0.778	0.009	0.011	0.008	0.93	0	0	100
15.0	3.75	3.750	0.882	0.883	0.001	0.001	0.009	0.92	0	0	100
20.0	3.75	3.750	0.973	0.966	-0.006	-0.006	0.014	0.90	0	0	100
2.5	5.00	3.750	0.532	0.563	0.031	0.059	0.017	0.91	0	1	99
5.0	5.00	3.750	0.671	0.695	0.024	0.035	0.010	0.95	0	0	100
7.5	5.00	3.750	0.769	0.787	0.018	0.023	0.009	0.94	0	0	100
10.0	5.00	3.750	0.848	0.860	0.012	0.014	0.008	0.93	0	0	100
15.0	5.00	3.750	0.972	0.974	0.002	0.002	0.009	0.92	0	0	100
20.0	5.00	3.750	1.071	1.065	-0.007	-0.006	0.014	0.91	0	0	100
2.5	7.50	3.750	0.610	0.659	0.049	0.080	0.023	0.87	0	0	100
5.0	7.50	3.750	0.770	0.807	0.037	0.048	0.013	0.89	0	0	100
7.5	7.50	3.750	0.882	0.910	0.028	0.032	0.010	0.92	0	0	100
10.0	7.50	3.750	0.972	0.992	0.020	0.021	0.010	0.94	0	0	100
15.0	7.50	3.750	1.114	1.121	0.007	0.006	0.012	0.93	0	0	100
20.0	7.50	3.750	1.228	1.222	-0.005	-0.004	0.019	0.92	0	0	100
2.5	10.00	3.750	0.673	0.738	0.065	0.097	0.034	0.82	0	0	100
5.0	10.00	3.750	0.848	0.898	0.050	0.059	0.021	0.87	0	0	100
7.5	10.00	3.750	0.972	1.010	0.038	0.039	0.018	0.92	0	0	100

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Table B.8 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
10.0	10.00	3.750	1.071	1.099	0.029	0.027	0.017	0.93	0	0	100
15.0	10.00	3.750	1.227	1.239	0.012	0.010	0.020	0.93	0	0	100
20.0	10.00	3.750	1.352	1.350	-0.002	-0.002	0.028	0.92	0	0	100
2.5	1.25	5.000	0.367	0.373	0.006	0.017	0.024	0.90	0	17	83
5.0	1.25	5.000	0.463	0.471	0.008	0.016	0.021	0.94	0	4	96
7.5	1.25	5.000	0.531	0.539	0.008	0.014	0.021	0.91	0	3	97
10.0	1.25	5.000	0.586	0.593	0.007	0.012	0.022	0.91	0	3	97
15.0	1.25	5.000	0.672	0.677	0.005	0.007	0.024	0.89	0	2	98
20.0	1.25	5.000	0.741	0.743	0.001	0.002	0.029	0.92	0	2	98
2.5	2.50	5.000	0.463	0.481	0.017	0.038	0.020	0.92	0	3	97
5.0	2.50	5.000	0.585	0.598	0.014	0.023	0.015	0.90	0	1	99
7.5	2.50	5.000	0.671	0.681	0.010	0.015	0.015	0.90	0	0	100
10.0	2.50	5.000	0.739	0.746	0.006	0.009	0.015	0.91	0	0	100
15.0	2.50	5.000	0.848	0.848	0.000	0.000	0.015	0.90	0	0	100
20.0	2.50	5.000	0.935	0.928	-0.007	-0.008	0.020	0.89	0	0	100
2.5	3.75	5.000	0.531	0.558	0.027	0.051	0.021	0.92	0	1	99
5.0	3.75	5.000	0.671	0.690	0.019	0.029	0.014	0.91	0	0	100
7.5	3.75	5.000	0.769	0.782	0.013	0.017	0.013	0.89	0	0	100
10.0	3.75	5.000	0.847	0.855	0.008	0.009	0.013	0.89	0	0	100
15.0	3.75	5.000	0.971	0.969	-0.002	-0.002	0.014	0.91	0	0	100
20.0	3.75	5.000	1.071	1.059	-0.012	-0.011	0.019	0.89	0	0	100
2.5	5.00	5.000	0.586	0.621	0.036	0.061	0.023	0.88	0	0	100
5.0	5.00	5.000	0.739	0.764	0.025	0.034	0.015	0.92	0	0	100
7.5	5.00	5.000	0.847	0.864	0.017	0.020	0.013	0.92	0	0	100
10.0	5.00	5.000	0.933	0.943	0.010	0.010	0.013	0.93	0	0	100
15.0	5.00	5.000	1.070	1.067	-0.003	-0.003	0.015	0.91	0	0	100
20.0	5.00	5.000	1.179	1.165	-0.014	-0.012	0.021	0.90	0	0	100
2.5	7.50	5.000	0.672	0.723	0.052	0.077	0.028	0.89	0	0	100
5.0	7.50	5.000	0.848	0.884	0.036	0.043	0.017	0.90	0	0	100
7.5	7.50	5.000	0.971	0.996	0.025	0.026	0.014	0.92	0	0	100
10.0	7.50	5.000	1.070	1.085	0.015	0.014	0.014	0.94	0	0	100
15.0	7.50	5.000	1.226	1.225	-0.001	-0.001	0.019	0.92	0	0	100
20.0	7.50	5.000	1.351	1.336	-0.016	-0.012	0.029	0.90	0	0	100
2.5	10.00	5.000	0.741	0.807	0.066	0.090	0.039	0.85	0	0	100
5.0	10.00	5.000	0.934	0.982	0.047	0.051	0.024	0.90	0	0	100
7.5	10.00	5.000	1.070	1.104	0.033	0.031	0.021	0.91	0	0	100
10.0	10.00	5.000	1.179	1.200	0.022	0.018	0.022	0.93	0	0	100
15.0	10.00	5.000	1.351	1.353	0.002	0.001	0.029	0.90	0	0	100
20.0	10.00	5.000	1.489	1.474	-0.015	-0.010	0.043	0.87	0	0	100

B.2 Model Function Goodness of Fit Tables, Second Scenario

B.2.1 Full Model

Table B.9 shows the detailed results from the evaluation of f_{12}, based on the full model. The function's constant true value of zero indicates additivity, and this is correctly identified by most simulation runs at most dose combinations.

Table B.9: Evaluation of f_{12} at all doses, for full model of Scenario 2.

DrugA	DrugB	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	1.25	0.000	-0.012	-0.012	.	0.009	0.95	5	95	0
5.0	1.25	0.000	-0.015	-0.015	.	0.006	0.97	3	97	0
7.5	1.25	0.000	-0.018	-0.018	.	0.007	0.96	4	96	0
10.0	1.25	0.000	-0.020	-0.020	.	0.009	0.93	6	93	1
15.0	1.25	0.000	-0.024	-0.024	.	0.014	0.94	5	94	1
20.0	1.25	0.000	-0.027	-0.027	.	0.023	0.93	6	93	1
2.5	2.50	0.000	-0.005	-0.005	.	0.006	0.96	3	96	1
5.0	2.50	0.000	-0.008	-0.008	.	0.003	0.96	2	96	2
7.5	2.50	0.000	-0.010	-0.010	.	0.004	0.96	2	96	2
10.0	2.50	0.000	-0.011	-0.011	.	0.005	0.95	4	95	1
15.0	2.50	0.000	-0.014	-0.014	.	0.009	0.92	6	92	2
20.0	2.50	0.000	-0.016	-0.016	.	0.016	0.92	8	92	0
2.5	3.75	0.000	-0.003	-0.003	.	0.007	0.95	2	95	3
5.0	3.75	0.000	-0.004	-0.004	.	0.004	0.94	3	94	3
7.5	3.75	0.000	-0.005	-0.005	.	0.004	0.96	3	96	1
10.0	3.75	0.000	-0.006	-0.006	.	0.005	0.95	4	95	1
15.0	3.75	0.000	-0.008	-0.008	.	0.008	0.94	5	94	1
20.0	3.75	0.000	-0.010	-0.010	.	0.014	0.94	5	94	1
2.5	5.00	0.000	-0.001	-0.001	.	0.008	0.94	3	94	3
5.0	5.00	0.000	-0.002	-0.002	.	0.005	0.94	3	94	3
7.5	5.00	0.000	-0.003	-0.003	.	0.005	0.95	4	95	1
10.0	5.00	0.000	-0.004	-0.004	.	0.006	0.93	6	93	1
15.0	5.00	0.000	-0.005	-0.005	.	0.008	0.92	7	92	1
20.0	5.00	0.000	-0.005	-0.005	.	0.013	0.94	5	94	1
2.5	7.50	0.000	-0.003	-0.003	.	0.013	0.95	4	95	1
5.0	7.50	0.000	-0.002	-0.002	.	0.009	0.93	5	93	2
7.5	7.50	0.000	-0.002	-0.002	.	0.009	0.91	6	91	3
10.0	7.50	0.000	-0.002	-0.002	.	0.009	0.92	6	92	2
15.0	7.50	0.000	-0.002	-0.002	.	0.009	0.92	6	92	2
20.0	7.50	0.000	-0.001	-0.001	.	0.013	0.95	4	95	1
2.5	10.00	0.000	-0.007	-0.007	.	0.023	0.95	4	95	1
5.0	10.00	0.000	-0.006	-0.006	.	0.017	0.94	4	94	2
7.5	10.00	0.000	-0.005	-0.005	.	0.016	0.93	5	93	2
10.0	10.00	0.000	-0.004	-0.004	.	0.015	0.92	5	92	3
15.0	10.00	0.000	-0.002	-0.002	.	0.014	0.94	4	94	2
20.0	10.00	0.000	-0.001	-0.001	.	0.016	0.95	4	95	1

Table B.10 shows the detailed results from the evaluation of f_{13}, based on the full model. The function's constant true value of zero indicates additivity, and this is correctly identified by most simulation runs at most dose combinations.

Table B.10: Evaluation of f_{13} at all doses, for full model of Scenario 2.

DrugA	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	0.625	0.000	-0.012	-0.012	.	0.008	0.97	1	97	2
5.0	0.625	0.000	-0.007	-0.007	.	0.007	0.96	3	96	1
7.5	0.625	0.000	-0.005	-0.005	.	0.008	0.97	2	97	1
10.0	0.625	0.000	-0.004	-0.004	.	0.010	0.96	3	96	1
15.0	0.625	0.000	-0.004	-0.004	.	0.017	0.95	4	95	1
20.0	0.625	0.000	-0.007	-0.007	.	0.031	0.92	6	92	2
2.5	1.250	0.000	-0.018	-0.018	.	0.006	0.94	5	94	1
5.0	1.250	0.000	-0.012	-0.012	.	0.004	0.91	5	91	4
7.5	1.250	0.000	-0.009	-0.009	.	0.005	0.93	4	93	3
10.0	1.250	0.000	-0.008	-0.008	.	0.007	0.91	7	91	2
15.0	1.250	0.000	-0.008	-0.008	.	0.013	0.89	9	89	2
20.0	1.250	0.000	-0.010	-0.010	.	0.025	0.90	8	90	2
2.5	1.875	0.000	-0.022	-0.022	.	0.007	0.92	7	92	1
5.0	1.875	0.000	-0.016	-0.016	.	0.005	0.88	8	88	4
7.5	1.875	0.000	-0.012	-0.012	.	0.006	0.90	5	90	5
10.0	1.875	0.000	-0.011	-0.011	.	0.008	0.90	7	90	3
15.0	1.875	0.000	-0.010	-0.010	.	0.013	0.86	11	86	3
20.0	1.875	0.000	-0.011	-0.011	.	0.022	0.89	10	89	1
2.5	2.500	0.000	-0.025	-0.025	.	0.009	0.93	7	93	0
5.0	2.500	0.000	-0.018	-0.018	.	0.006	0.89	8	89	3
7.5	2.500	0.000	-0.014	-0.014	.	0.007	0.93	4	93	3
10.0	2.500	0.000	-0.012	-0.012	.	0.008	0.90	6	90	4
15.0	2.500	0.000	-0.011	-0.011	.	0.012	0.89	8	89	3
20.0	2.500	0.000	-0.012	-0.012	.	0.021	0.89	9	89	2
2.5	3.750	0.000	-0.029	-0.029	.	0.014	0.92	7	92	1
5.0	3.750	0.000	-0.022	-0.022	.	0.009	0.92	6	92	2
7.5	3.750	0.000	-0.017	-0.017	.	0.009	0.96	3	96	1
10.0	3.750	0.000	-0.015	-0.015	.	0.009	0.94	5	94	1
15.0	3.750	0.000	-0.013	-0.013	.	0.012	0.91	6	91	3
20.0	3.750	0.000	-0.014	-0.014	.	0.018	0.89	9	89	2
2.5	5.000	0.000	-0.033	-0.033	.	0.024	0.93	6	93	1
5.0	5.000	0.000	-0.024	-0.024	.	0.016	0.94	5	94	1
7.5	5.000	0.000	-0.020	-0.020	.	0.014	0.95	4	95	1
10.0	5.000	0.000	-0.017	-0.017	.	0.013	0.94	4	94	2
15.0	5.000	0.000	-0.014	-0.014	.	0.014	0.95	4	95	1
20.0	5.000	0.000	-0.014	-0.014	.	0.019	0.91	7	91	2

Table B.11 shows the detailed results from the evaluation of f_{23}, based on the full model. The function's constant true value of zero indicates additivity, and this is correctly identified by most simulation runs at most dose combinations.

Table B.11: Evaluation of f_{23} at all doses, for full model of Scenario 2.

DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
1.25	0.625	0.000	-0.011	-0.011	.	0.014	0.97	2	97	1
2.50	0.625	0.000	-0.011	-0.011	.	0.012	0.97	2	97	1
3.75	0.625	0.000	-0.013	-0.013	.	0.014	0.93	5	93	2
5.00	0.625	0.000	-0.015	-0.015	.	0.016	0.94	5	94	1
7.50	0.625	0.000	-0.019	-0.019	.	0.023	0.89	8	89	3
10.00	0.625	0.000	-0.025	-0.025	.	0.038	0.87	10	87	3
1.25	1.250	0.000	-0.011	-0.011	.	0.008	0.97	2	97	1
2.50	1.250	0.000	-0.010	-0.010	.	0.007	0.94	5	94	1
3.75	1.250	0.000	-0.010	-0.010	.	0.007	0.92	7	92	1
5.00	1.250	0.000	-0.011	-0.011	.	0.009	0.91	7	91	2
7.50	1.250	0.000	-0.013	-0.013	.	0.013	0.90	7	90	3
10.00	1.250	0.000	-0.017	-0.017	.	0.022	0.91	7	91	2
1.25	1.875	0.000	-0.013	-0.013	.	0.008	0.96	3	96	1
2.50	1.875	0.000	-0.011	-0.011	.	0.007	0.94	5	94	1
3.75	1.875	0.000	-0.010	-0.010	.	0.007	0.93	7	93	0
5.00	1.875	0.000	-0.010	-0.010	.	0.007	0.92	8	92	0
7.50	1.875	0.000	-0.011	-0.011	.	0.010	0.91	8	91	1
10.00	1.875	0.000	-0.013	-0.013	.	0.017	0.91	7	91	2
1.25	2.500	0.000	-0.016	-0.016	.	0.009	0.94	5	94	1
2.50	2.500	0.000	-0.013	-0.013	.	0.007	0.93	6	93	1
3.75	2.500	0.000	-0.011	-0.011	.	0.007	0.92	8	92	0
5.00	2.500	0.000	-0.010	-0.010	.	0.007	0.93	7	93	0
7.50	2.500	0.000	-0.010	-0.010	.	0.009	0.92	7	92	1
10.00	2.500	0.000	-0.011	-0.011	.	0.015	0.93	6	93	1
1.25	3.750	0.000	-0.024	-0.024	.	0.012	0.96	4	96	0
2.50	3.750	0.000	-0.019	-0.019	.	0.009	0.95	5	95	0
3.75	3.750	0.000	-0.015	-0.015	.	0.008	0.91	9	91	0
5.00	3.750	0.000	-0.013	-0.013	.	0.008	0.93	7	93	0
7.50	3.750	0.000	-0.011	-0.011	.	0.009	0.91	8	91	1
10.00	3.750	0.000	-0.011	-0.011	.	0.013	0.91	7	91	2
1.25	5.000	0.000	-0.034	-0.034	.	0.020	0.95	5	95	0
2.50	5.000	0.000	-0.027	-0.027	.	0.015	0.93	7	93	0
3.75	5.000	0.000	-0.022	-0.022	.	0.013	0.91	8	91	1
5.00	5.000	0.000	-0.019	-0.019	.	0.013	0.92	7	92	1
7.50	5.000	0.000	-0.015	-0.015	.	0.013	0.89	10	89	1
10.00	5.000	0.000	-0.013	-0.013	.	0.017	0.90	9	90	1

Table B.12 shows the detailed results from the evaluation of f_{123}, based on the full model. The true value of the function has some positive values and some negative values, reflecting the mix of synergism and antagonism. The sign of the value of the function was correctly estimated by most simulation runs, and the correct relationship (synergism or antagonism) was identified in many cases. In some cases the magnitude of the relationship did not reach statistical significance, so some cases of true synergism

or true antagonism were identified as additive.

Table B.12: Evaluation of f_{123} at all doses, for full model of Scenario 2.

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	1.25	0.625	1.160	1.190	0.031	0.027	0.041	0.95	0	0	100
5.0	1.25	0.625	1.039	1.072	0.032	0.031	0.030	0.93	0	0	100
7.5	1.25	0.625	0.954	0.987	0.033	0.034	0.027	0.96	0	0	100
10.0	1.25	0.625	0.886	0.919	0.032	0.036	0.025	0.95	0	0	100
15.0	1.25	0.625	0.779	0.809	0.030	0.039	0.026	0.93	0	1	99
20.0	1.25	0.625	0.692	0.719	0.027	0.039	0.039	0.92	0	4	96
2.5	2.50	0.625	1.039	1.059	0.020	0.019	0.034	0.92	0	0	100
5.0	2.50	0.625	0.888	0.909	0.021	0.024	0.025	0.91	0	0	100
7.5	2.50	0.625	0.781	0.802	0.022	0.028	0.021	0.94	0	0	100
10.0	2.50	0.625	0.695	0.717	0.022	0.031	0.019	0.92	0	0	100
15.0	2.50	0.625	0.559	0.579	0.020	0.035	0.020	0.98	0	1	99
20.0	2.50	0.625	0.451	0.468	0.017	0.037	0.032	0.95	0	28	72
2.5	3.75	0.625	0.954	0.969	0.014	0.015	0.033	0.92	0	0	100
5.0	3.75	0.625	0.781	0.797	0.016	0.021	0.024	0.91	0	0	100
7.5	3.75	0.625	0.658	0.675	0.017	0.026	0.022	0.94	0	0	100
10.0	3.75	0.625	0.561	0.577	0.017	0.030	0.020	0.94	0	0	100
15.0	3.75	0.625	0.405	0.420	0.015	0.037	0.022	0.96	0	21	79
20.0	3.75	0.625	0.281	0.294	0.013	0.045	0.035	0.94	0	71	29
2.5	5.00	0.625	0.887	0.899	0.012	0.014	0.033	0.92	0	0	100
5.0	5.00	0.625	0.695	0.709	0.014	0.020	0.025	0.92	0	0	100
7.5	5.00	0.625	0.561	0.575	0.015	0.026	0.023	0.94	0	1	99
10.0	5.00	0.625	0.453	0.468	0.015	0.033	0.022	0.93	0	9	91
15.0	5.00	0.625	0.282	0.296	0.013	0.048	0.024	0.96	0	55	45
20.0	5.00	0.625	0.146	0.157	0.011	0.076	0.039	0.95	0	87	13
2.5	7.50	0.625	0.779	0.792	0.013	0.016	0.036	0.93	0	0	100
5.0	7.50	0.625	0.560	0.575	0.015	0.027	0.029	0.94	0	3	97
7.5	7.50	0.625	0.406	0.421	0.016	0.039	0.028	0.94	0	20	80
10.0	7.50	0.625	0.282	0.298	0.016	0.056	0.028	0.93	0	52	48
15.0	7.50	0.625	0.087	0.102	0.015	0.172	0.034	0.95	0	88	12
20.0	7.50	0.625	-0.069	-0.056	0.013	-0.186	0.052	0.94	4	93	3
2.5	10.00	0.625	0.693	0.711	0.018	0.026	0.052	0.96	0	9	91
5.0	10.00	0.625	0.452	0.472	0.020	0.044	0.044	0.93	0	38	62
7.5	10.00	0.625	0.282	0.303	0.021	0.074	0.045	0.91	0	61	39
10.0	10.00	0.625	0.146	0.167	0.021	0.145	0.047	0.93	0	85	15
15.0	10.00	0.625	-0.069	-0.048	0.021	-0.297	0.057	0.93	3	95	2
20.0	10.00	0.625	-0.241	-0.222	0.019	-0.078	0.079	0.94	13	86	1
2.5	1.25	1.250	1.201	1.229	0.028	0.023	0.027	0.98	0	0	100
5.0	1.25	1.250	1.049	1.079	0.029	0.028	0.019	0.98	0	0	100
7.5	1.25	1.250	0.942	0.972	0.030	0.032	0.017	0.99	0	0	100
10.0	1.25	1.250	0.857	0.886	0.029	0.034	0.017	0.97	0	0	100
15.0	1.25	1.250	0.721	0.749	0.028	0.038	0.022	0.93	0	0	100
20.0	1.25	1.250	0.612	0.637	0.025	0.040	0.039	0.89	0	9	91
2.5	2.50	1.250	1.049	1.066	0.016	0.016	0.020	0.97	0	0	100
5.0	2.50	1.250	0.858	0.877	0.018	0.021	0.012	0.96	0	0	100
7.5	2.50	1.250	0.724	0.742	0.019	0.026	0.010	0.96	0	0	100
10.0	2.50	1.250	0.616	0.634	0.019	0.030	0.010	0.97	0	0	100
15.0	2.50	1.250	0.445	0.462	0.017	0.038	0.014	0.95	0	3	97
20.0	2.50	1.250	0.308	0.322	0.014	0.047	0.030	0.93	0	51	49
2.5	3.75	1.250	0.942	0.953	0.011	0.012	0.020	0.97	0	0	100
5.0	3.75	1.250	0.724	0.737	0.013	0.018	0.011	0.96	0	0	100
7.5	3.75	1.250	0.569	0.583	0.014	0.024	0.010	0.93	0	0	100
10.0	3.75	1.250	0.446	0.460	0.014	0.031	0.010	0.98	0	1	99
15.0	3.75	1.250	0.250	0.263	0.012	0.049	0.014	0.98	0	42	58
20.0	3.75	1.250	0.094	0.104	0.010	0.105	0.031	0.93	0	88	12
2.5	5.00	1.250	0.857	0.866	0.009	0.010	0.019	0.97	0	0	100

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Table B.12 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
5.0	5.00	1.250	0.616	0.627	0.011	0.018	0.011	0.93	0	0	100
7.5	5.00	1.250	0.446	0.458	0.012	0.026	0.010	0.95	0	1	99
10.0	5.00	1.250	0.311	0.322	0.012	0.037	0.011	0.96	0	19	81
15.0	5.00	1.250	0.095	0.106	0.010	0.110	0.016	0.95	0	84	16
20.0	5.00	1.250	-0.077	-0.068	0.008	-0.108	0.034	0.93	3	94	3
2.5	7.50	1.250	0.721	0.731	0.009	0.013	0.022	0.95	0	0	100
5.0	7.50	1.250	0.445	0.457	0.012	0.026	0.014	0.94	0	3	97
7.5	7.50	1.250	0.251	0.263	0.012	0.049	0.014	0.91	0	44	56
10.0	7.50	1.250	0.096	0.108	0.013	0.132	0.016	0.96	0	83	17
15.0	7.50	1.250	-0.151	-0.139	0.012	-0.078	0.024	0.92	14	86	0
20.0	7.50	1.250	-0.348	-0.338	0.010	-0.029	0.045	0.93	37	63	0
2.5	10.00	1.250	0.613	0.627	0.014	0.023	0.037	0.96	0	11	89
5.0	10.00	1.250	0.309	0.325	0.017	0.054	0.029	0.96	0	50	50
7.5	10.00	1.250	0.095	0.112	0.018	0.185	0.029	0.94	0	89	11
10.0	10.00	1.250	-0.076	-0.058	0.018	-0.235	0.032	0.94	5	93	2
15.0	10.00	1.250	-0.347	-0.330	0.017	-0.050	0.044	0.93	37	63	0
20.0	10.00	1.250	-0.564	-0.548	0.016	-0.028	0.069	0.92	65	35	0
2.5	1.25	1.875	1.230	1.258	0.028	0.023	0.027	0.96	0	0	100
5.0	1.25	1.875	1.057	1.086	0.030	0.028	0.018	0.95	0	0	100
7.5	1.25	1.875	0.934	0.964	0.030	0.032	0.017	0.97	0	0	100
10.0	1.25	1.875	0.836	0.866	0.030	0.036	0.018	0.95	0	0	100
15.0	1.25	1.875	0.681	0.709	0.028	0.041	0.024	0.92	0	0	100
20.0	1.25	1.875	0.556	0.582	0.025	0.046	0.043	0.90	0	16	84
2.5	2.50	1.875	1.057	1.073	0.016	0.016	0.020	0.97	0	0	100
5.0	2.50	1.875	0.838	0.856	0.018	0.022	0.011	0.93	0	0	100
7.5	2.50	1.875	0.683	0.702	0.019	0.028	0.010	0.95	0	0	100
10.0	2.50	1.875	0.560	0.579	0.019	0.034	0.010	0.95	0	0	100
15.0	2.50	1.875	0.364	0.382	0.018	0.048	0.016	0.94	0	16	84
20.0	2.50	1.875	0.208	0.223	0.015	0.073	0.033	0.92	0	70	30
2.5	3.75	1.875	0.934	0.945	0.011	0.012	0.019	0.97	0	0	100
5.0	3.75	1.875	0.683	0.697	0.013	0.019	0.010	0.93	0	0	100
7.5	3.75	1.875	0.507	0.521	0.014	0.027	0.009	0.95	0	0	100
10.0	3.75	1.875	0.366	0.380	0.014	0.038	0.010	0.97	0	7	93
15.0	3.75	1.875	0.142	0.155	0.013	0.090	0.016	0.95	0	72	28
20.0	3.75	1.875	-0.037	-0.027	0.010	-0.282	0.033	0.92	2	94	4
2.5	5.00	1.875	0.836	0.845	0.009	0.010	0.019	0.98	0	0	100
5.0	5.00	1.875	0.560	0.571	0.011	0.019	0.010	0.96	0	0	100
7.5	5.00	1.875	0.366	0.378	0.012	0.032	0.009	0.94	0	4	96
10.0	5.00	1.875	0.211	0.223	0.012	0.056	0.010	0.95	0	41	59
15.0	5.00	1.875	-0.036	-0.025	0.011	-0.303	0.017	0.94	2	96	2
20.0	5.00	1.875	-0.233	-0.224	0.009	-0.038	0.036	0.92	21	79	0
2.5	7.50	1.875	0.681	0.690	0.009	0.014	0.021	0.96	0	0	100
5.0	7.50	1.875	0.365	0.376	0.012	0.032	0.013	0.96	0	8	92
7.5	7.50	1.875	0.142	0.155	0.012	0.087	0.013	0.93	0	71	29
10.0	7.50	1.875	-0.035	-0.023	0.013	-0.359	0.015	0.95	3	94	3
15.0	7.50	1.875	-0.317	-0.305	0.012	-0.038	0.025	0.90	55	45	0
20.0	7.50	1.875	-0.543	-0.532	0.010	-0.019	0.047	0.92	80	20	0
2.5	10.00	1.875	0.557	0.571	0.014	0.026	0.036	0.96	0	14	86
5.0	10.00	1.875	0.209	0.225	0.016	0.079	0.026	0.93	0	73	27
7.5	10.00	1.875	-0.036	-0.019	0.017	-0.481	0.027	0.94	2	94	4
10.0	10.00	1.875	-0.232	-0.214	0.018	-0.077	0.030	0.91	25	75	0
15.0	10.00	1.875	-0.542	-0.525	0.017	-0.032	0.044	0.92	80	20	0
20.0	10.00	1.875	-0.790	-0.774	0.016	-0.020	0.070	0.92	95	5	0
2.5	1.25	2.500	1.254	1.283	0.030	0.024	0.027	0.96	0	0	100
5.0	1.25	2.500	1.062	1.094	0.032	0.030	0.019	0.96	0	0	100
7.5	1.25	2.500	0.927	0.960	0.032	0.035	0.018	0.95	0	0	100
10.0	1.25	2.500	0.820	0.852	0.032	0.039	0.019	0.95	0	0	100
15.0	1.25	2.500	0.648	0.679	0.030	0.047	0.026	0.93	0	0	100

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Table B.12 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
20.0	1.25	2.500	0.512	0.539	0.028	0.054	0.045	0.89	0	19	81
2.5	2.50	2.500	1.062	1.080	0.018	0.017	0.021	0.97	0	0	100
5.0	2.50	2.500	0.821	0.842	0.020	0.025	0.012	0.95	0	0	100
7.5	2.50	2.500	0.651	0.672	0.021	0.032	0.010	0.95	0	0	100
10.0	2.50	2.500	0.516	0.537	0.021	0.041	0.011	0.94	0	0	100
15.0	2.50	2.500	0.300	0.320	0.020	0.065	0.017	0.91	0	26	74
20.0	2.50	2.500	0.128	0.145	0.017	0.136	0.034	0.90	0	79	21
2.5	3.75	2.500	0.927	0.940	0.013	0.014	0.020	0.96	0	0	100
5.0	3.75	2.500	0.652	0.666	0.015	0.023	0.011	0.91	0	0	100
7.5	3.75	2.500	0.457	0.473	0.016	0.034	0.010	0.96	0	0	100
10.0	3.75	2.500	0.302	0.318	0.016	0.052	0.010	0.93	0	13	87
15.0	3.75	2.500	0.055	0.070	0.015	0.267	0.017	0.91	0	85	15
20.0	3.75	2.500	-0.142	-0.129	0.013	-0.090	0.035	0.90	8	91	1
2.5	5.00	2.500	0.820	0.830	0.010	0.013	0.020	0.95	0	0	100
5.0	5.00	2.500	0.516	0.529	0.013	0.024	0.011	0.96	0	0	100
7.5	5.00	2.500	0.302	0.315	0.013	0.044	0.010	0.94	0	15	85
10.0	5.00	2.500	0.131	0.145	0.014	0.104	0.011	0.92	0	70	30
15.0	5.00	2.500	-0.140	-0.127	0.013	-0.091	0.018	0.93	15	85	0
20.0	5.00	2.500	-0.357	-0.346	0.011	-0.031	0.037	0.91	48	52	0
2.5	7.50	2.500	0.649	0.660	0.011	0.017	0.022	0.97	0	0	100
5.0	7.50	2.500	0.301	0.314	0.013	0.044	0.013	0.96	0	20	80
7.5	7.50	2.500	0.056	0.070	0.014	0.254	0.013	0.94	0	89	11
10.0	7.50	2.500	-0.140	-0.125	0.014	-0.103	0.015	0.95	11	88	1
15.0	7.50	2.500	-0.450	-0.436	0.014	-0.031	0.025	0.88	85	15	0
20.0	7.50	2.500	-0.698	-0.686	0.012	-0.018	0.048	0.90	97	3	0
2.5	10.00	2.500	0.512	0.528	0.016	0.031	0.037	0.96	0	22	78
5.0	10.00	2.500	0.129	0.147	0.018	0.141	0.026	0.95	0	86	14
7.5	10.00	2.500	-0.141	-0.122	0.019	-0.136	0.026	0.94	8	91	1
10.0	10.00	2.500	-0.356	-0.337	0.020	-0.055	0.030	0.94	54	46	0
15.0	10.00	2.500	-0.698	-0.679	0.019	-0.027	0.043	0.91	96	4	0
20.0	10.00	2.500	-0.971	-0.953	0.018	-0.018	0.070	0.92	100	0	0
2.5	1.25	3.750	1.291	1.327	0.036	0.028	0.032	0.96	0	0	100
5.0	1.25	3.750	1.071	1.110	0.038	0.036	0.022	0.97	0	0	100
7.5	1.25	3.750	0.917	0.956	0.039	0.042	0.020	0.97	0	0	100
10.0	1.25	3.750	0.793	0.832	0.039	0.049	0.020	0.95	0	0	100
15.0	1.25	3.750	0.597	0.635	0.038	0.063	0.026	0.92	0	2	98
20.0	1.25	3.750	0.441	0.476	0.035	0.080	0.044	0.91	0	32	68
2.5	2.50	3.750	1.071	1.096	0.025	0.023	0.026	0.93	0	0	100
5.0	2.50	3.750	0.795	0.822	0.027	0.034	0.016	0.92	0	0	100
7.5	2.50	3.750	0.601	0.629	0.028	0.046	0.013	0.92	0	0	100
10.0	2.50	3.750	0.446	0.473	0.028	0.062	0.013	0.95	0	1	99
15.0	2.50	3.750	0.199	0.225	0.027	0.134	0.018	0.93	0	55	45
20.0	2.50	3.750	0.002	0.026	0.025	15.172	0.034	0.90	1	90	9
2.5	3.75	3.750	0.917	0.936	0.019	0.021	0.026	0.94	0	0	100
5.0	3.75	3.750	0.601	0.622	0.021	0.036	0.015	0.92	0	0	100
7.5	3.75	3.750	0.378	0.401	0.022	0.059	0.013	0.90	0	5	95
10.0	3.75	3.750	0.201	0.223	0.022	0.112	0.013	0.92	0	43	57
15.0	3.75	3.750	-0.082	-0.060	0.022	-0.266	0.018	0.92	4	93	3
20.0	3.75	3.750	-0.307	-0.287	0.020	-0.065	0.035	0.91	35	65	0
2.5	5.00	3.750	0.793	0.810	0.017	0.021	0.026	0.93	0	0	100
5.0	5.00	3.750	0.446	0.465	0.019	0.043	0.015	0.90	0	1	99
7.5	5.00	3.750	0.201	0.221	0.020	0.099	0.013	0.91	0	43	57
10.0	5.00	3.750	0.005	0.025	0.020	3.911	0.013	0.90	1	88	11
15.0	5.00	3.750	-0.305	-0.286	0.020	-0.064	0.020	0.91	57	43	0
20.0	5.00	3.750	-0.554	-0.536	0.018	-0.032	0.038	0.91	88	12	0
2.5	7.50	3.750	0.598	0.615	0.017	0.029	0.028	0.91	0	2	98
5.0	7.50	3.750	0.199	0.219	0.020	0.098	0.016	0.92	0	52	48
7.5	7.50	3.750	-0.081	-0.061	0.021	-0.253	0.015	0.93	7	92	1

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Table B.12 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
10.0	7.50	3.750	-0.305	-0.284	0.021	-0.069	0.017	0.90	70	30	0
15.0	7.50	3.750	-0.660	-0.640	0.021	-0.031	0.026	0.90	100	0	0
20.0	7.50	3.750	-0.944	-0.925	0.019	-0.020	0.049	0.92	100	0	0
2.5	10.00	3.750	0.441	0.463	0.022	0.050	0.041	0.95	0	42	58
5.0	10.00	3.750	0.002	0.027	0.024	10.681	0.028	0.95	0	95	5
7.5	10.00	3.750	-0.306	-0.281	0.025	-0.083	0.027	0.94	39	61	0
10.0	10.00	3.750	-0.553	-0.527	0.026	-0.047	0.030	0.95	92	8	0
15.0	10.00	3.750	-0.944	-0.918	0.026	-0.027	0.044	0.93	100	0	0
20.0	10.00	3.750	-1.256	-1.232	0.025	-0.020	0.071	0.92	100	0	0
2.5	1.25	5.000	1.320	1.365	0.045	0.034	0.048	0.93	0	0	100
5.0	1.25	5.000	1.079	1.126	0.047	0.044	0.036	0.93	0	0	100
7.5	1.25	5.000	0.909	0.957	0.048	0.053	0.032	0.95	0	0	100
10.0	1.25	5.000	0.773	0.821	0.048	0.062	0.031	0.94	0	0	100
15.0	1.25	5.000	0.557	0.604	0.047	0.085	0.034	0.96	0	11	89
20.0	1.25	5.000	0.384	0.430	0.045	0.117	0.049	0.95	0	46	54
2.5	2.50	5.000	1.079	1.112	0.034	0.031	0.043	0.91	0	0	100
5.0	2.50	5.000	0.775	0.811	0.036	0.046	0.030	0.93	0	0	100
7.5	2.50	5.000	0.561	0.597	0.037	0.065	0.026	0.92	0	2	98
10.0	2.50	5.000	0.390	0.426	0.037	0.095	0.024	0.93	0	17	83
15.0	2.50	5.000	0.118	0.154	0.036	0.306	0.026	0.94	0	83	17
20.0	2.50	5.000	-0.099	-0.065	0.034	-0.345	0.040	0.95	5	92	3
2.5	3.75	5.000	0.908	0.937	0.028	0.031	0.043	0.92	0	0	100
5.0	3.75	5.000	0.561	0.591	0.030	0.054	0.030	0.91	0	2	98
7.5	3.75	5.000	0.316	0.347	0.031	0.099	0.026	0.91	0	27	73
10.0	3.75	5.000	0.120	0.152	0.032	0.263	0.024	0.93	0	80	20
15.0	3.75	5.000	-0.191	-0.160	0.031	-0.162	0.027	0.93	17	82	1
20.0	3.75	5.000	-0.439	-0.410	0.029	-0.067	0.042	0.95	49	51	0
2.5	5.00	5.000	0.773	0.798	0.026	0.033	0.043	0.92	0	1	99
5.0	5.00	5.000	0.390	0.418	0.028	0.072	0.030	0.91	0	17	83
7.5	5.00	5.000	0.120	0.149	0.029	0.241	0.026	0.90	1	75	24
10.0	5.00	5.000	-0.095	-0.066	0.029	-0.307	0.025	0.91	8	88	4
15.0	5.00	5.000	-0.437	-0.408	0.029	-0.066	0.029	0.93	74	26	0
20.0	5.00	5.000	-0.710	-0.683	0.027	-0.038	0.046	0.95	95	5	0
2.5	7.50	5.000	0.557	0.583	0.026	0.047	0.045	0.91	0	16	84
5.0	7.50	5.000	0.118	0.147	0.028	0.240	0.031	0.89	1	80	19
7.5	7.50	5.000	-0.190	-0.161	0.029	-0.154	0.028	0.88	18	81	1
10.0	7.50	5.000	-0.437	-0.407	0.030	-0.068	0.028	0.90	81	19	0
15.0	7.50	5.000	-0.828	-0.798	0.030	-0.036	0.036	0.92	100	0	0
20.0	7.50	5.000	-1.140	-1.112	0.028	-0.025	0.057	0.95	100	0	0
2.5	10.00	5.000	0.385	0.416	0.031	0.081	0.057	0.93	0	56	44
5.0	10.00	5.000	-0.099	-0.065	0.033	-0.337	0.041	0.93	8	91	1
7.5	10.00	5.000	-0.438	-0.404	0.034	-0.078	0.038	0.93	60	40	0
10.0	10.00	5.000	-0.710	-0.675	0.035	-0.049	0.040	0.93	95	5	0
15.0	10.00	5.000	-1.140	-1.105	0.035	-0.030	0.053	0.93	100	0	0
20.0	10.00	5.000	-1.484	-1.450	0.034	-0.023	0.078	0.94	100	0	0

B.2.2 Final Model

Table B.13 shows the detailed results from the evaluation of f_{12}, based on the final model. The function's constant true value of zero indicates additivity, and this is correctly identified by most simulation runs at most dose combinations.

Table B.13: Evaluation of f_{12} at all doses, for final model of Scenario 2.

DrugA	DrugB	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	1.25	0.000	-0.006	-0.006	.	0.005	0.92	4	92	4
5.0	1.25	0.000	-0.013	-0.013	.	0.004	0.93	4	93	3
7.5	1.25	0.000	-0.018	-0.018	.	0.005	0.89	8	89	3
10.0	1.25	0.000	-0.021	-0.021	.	0.006	0.87	9	87	4
15.0	1.25	0.000	-0.024	-0.024	.	0.009	0.87	9	87	4
20.0	1.25	0.000	-0.024	-0.024	.	0.014	0.85	10	85	5
2.5	2.50	0.000	-0.004	-0.004	.	0.004	0.93	3	93	4
5.0	2.50	0.000	-0.010	-0.010	.	0.003	0.96	2	96	2
7.5	2.50	0.000	-0.013	-0.013	.	0.004	0.93	4	93	3
10.0	2.50	0.000	-0.015	-0.015	.	0.004	0.91	5	91	4
15.0	2.50	0.000	-0.017	-0.017	.	0.007	0.89	7	89	4
20.0	2.50	0.000	-0.017	-0.017	.	0.011	0.86	9	86	5
2.5	3.75	0.000	-0.002	-0.002	.	0.005	0.94	2	94	4
5.0	3.75	0.000	-0.008	-0.008	.	0.003	0.93	2	93	5
7.5	3.75	0.000	-0.010	-0.010	.	0.004	0.91	5	91	4
10.0	3.75	0.000	-0.012	-0.012	.	0.005	0.89	6	89	5
15.0	3.75	0.000	-0.013	-0.013	.	0.006	0.89	6	89	5
20.0	3.75	0.000	-0.012	-0.012	.	0.010	0.89	6	89	5
2.5	5.00	0.000	-0.002	-0.002	.	0.006	0.90	5	90	5
5.0	5.00	0.000	-0.007	-0.007	.	0.004	0.91	3	91	6
7.5	5.00	0.000	-0.009	-0.009	.	0.004	0.89	6	89	5
10.0	5.00	0.000	-0.010	-0.010	.	0.005	0.89	6	89	5
15.0	5.00	0.000	-0.010	-0.010	.	0.007	0.88	6	88	6
20.0	5.00	0.000	-0.008	-0.008	.	0.010	0.89	6	89	5
2.5	7.50	0.000	-0.003	-0.003	.	0.009	0.89	5	89	6
5.0	7.50	0.000	-0.006	-0.006	.	0.006	0.89	5	89	6
7.5	7.50	0.000	-0.008	-0.008	.	0.006	0.89	6	89	5
10.0	7.50	0.000	-0.008	-0.008	.	0.006	0.89	5	89	6
15.0	7.50	0.000	-0.007	-0.007	.	0.007	0.88	6	88	6
20.0	7.50	0.000	-0.004	-0.004	.	0.010	0.89	5	89	6
2.5	10.00	0.000	-0.006	-0.006	.	0.014	0.86	7	86	7
5.0	10.00	0.000	-0.008	-0.008	.	0.010	0.88	6	88	6
7.5	10.00	0.000	-0.008	-0.008	.	0.008	0.88	8	88	4
10.0	10.00	0.000	-0.008	-0.008	.	0.008	0.87	8	87	5
15.0	10.00	0.000	-0.005	-0.005	.	0.009	0.89	5	89	6
20.0	10.00	0.000	-0.002	-0.002	.	0.011	0.89	5	89	6

Table B.14 shows the detailed results from the evaluation of f_{13}, based on the final model. The function's constant true value of zero indicates additivity, and this is correctly identified by most simulation runs at most dose combinations.

Table B.15 shows the detailed results from the evaluation of f_{23}, based on the final

Table B.14: Evaluation of f_{13} at all doses, for final model of Scenario 2.

DrugA	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	0.625	0.000	-0.016	-0.016	.	0.006	0.91	6	91	3
5.0	0.625	0.000	-0.010	-0.010	.	0.005	0.89	7	89	4
7.5	0.625	0.000	-0.008	-0.008	.	0.007	0.91	6	91	3
10.0	0.625	0.000	-0.008	-0.008	.	0.008	0.85	9	85	6
15.0	0.625	0.000	-0.012	-0.012	.	0.013	0.79	13	79	8
20.0	0.625	0.000	-0.019	-0.019	.	0.022	0.76	15	76	9
2.5	1.250	0.000	-0.017	-0.017	.	0.005	0.89	8	89	3
5.0	1.250	0.000	-0.010	-0.010	.	0.004	0.89	7	89	4
7.5	1.250	0.000	-0.008	-0.008	.	0.004	0.92	5	92	3
10.0	1.250	0.000	-0.007	-0.007	.	0.006	0.88	8	88	4
15.0	1.250	0.000	-0.010	-0.010	.	0.009	0.83	12	83	5
20.0	1.250	0.000	-0.016	-0.016	.	0.016	0.79	13	79	8
2.5	1.875	0.000	-0.019	-0.019	.	0.006	0.89	9	89	2
5.0	1.875	0.000	-0.012	-0.012	.	0.004	0.89	8	89	3
7.5	1.875	0.000	-0.009	-0.009	.	0.005	0.91	5	91	4
10.0	1.875	0.000	-0.008	-0.008	.	0.006	0.86	9	86	5
15.0	1.875	0.000	-0.011	-0.011	.	0.009	0.81	13	81	6
20.0	1.875	0.000	-0.016	-0.016	.	0.015	0.76	15	76	9
2.5	2.500	0.000	-0.022	-0.022	.	0.007	0.84	12	84	4
5.0	2.500	0.000	-0.014	-0.014	.	0.005	0.86	11	86	3
7.5	2.500	0.000	-0.011	-0.011	.	0.005	0.88	8	88	4
10.0	2.500	0.000	-0.010	-0.010	.	0.006	0.85	10	85	5
15.0	2.500	0.000	-0.012	-0.012	.	0.009	0.80	14	80	6
20.0	2.500	0.000	-0.017	-0.017	.	0.014	0.77	15	77	8
2.5	3.750	0.000	-0.029	-0.029	.	0.010	0.83	13	83	4
5.0	3.750	0.000	-0.020	-0.020	.	0.008	0.84	12	84	4
7.5	3.750	0.000	-0.016	-0.016	.	0.007	0.88	8	88	4
10.0	3.750	0.000	-0.015	-0.015	.	0.008	0.88	8	88	4
15.0	3.750	0.000	-0.016	-0.016	.	0.009	0.81	13	81	6
20.0	3.750	0.000	-0.020	-0.020	.	0.014	0.76	16	76	8
2.5	5.000	0.000	-0.037	-0.037	.	0.017	0.78	16	78	6
5.0	5.000	0.000	-0.027	-0.027	.	0.013	0.80	14	80	6
7.5	5.000	0.000	-0.023	-0.023	.	0.012	0.83	11	83	6
10.0	5.000	0.000	-0.021	-0.021	.	0.011	0.82	11	82	7
15.0	5.000	0.000	-0.021	-0.021	.	0.013	0.79	13	79	8
20.0	5.000	0.000	-0.025	-0.025	.	0.017	0.77	15	77	8

model. The function's constant true value of zero indicates additivity, and this is correctly identified by most simulation runs at most dose combinations.

Table B.15: Evaluation of f_{23} at all doses, for final model of Scenario 2.

DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
1.25	0.625	0.000	-0.008	-0.008	.	0.012	0.85	11	85	4
2.50	0.625	0.000	-0.009	-0.009	.	0.010	0.88	8	88	4
3.75	0.625	0.000	-0.011	-0.011	.	0.011	0.84	11	84	5
5.00	0.625	0.000	-0.013	-0.013	.	0.012	0.83	12	83	5
7.50	0.625	0.000	-0.017	-0.017	.	0.015	0.78	17	78	5
10.00	0.625	0.000	-0.021	-0.021	.	0.020	0.78	15	78	7
1.25	1.250	0.000	-0.008	-0.008	.	0.007	0.86	9	86	5
2.50	1.250	0.000	-0.008	-0.008	.	0.006	0.89	7	89	4
3.75	1.250	0.000	-0.009	-0.009	.	0.006	0.89	8	89	3
5.00	1.250	0.000	-0.010	-0.010	.	0.007	0.87	9	87	4
7.50	1.250	0.000	-0.013	-0.013	.	0.009	0.81	15	81	4
10.00	1.250	0.000	-0.017	-0.017	.	0.013	0.80	14	80	6
1.25	1.875	0.000	-0.009	-0.009	.	0.007	0.90	8	90	2
2.50	1.875	0.000	-0.009	-0.009	.	0.006	0.90	8	90	2
3.75	1.875	0.000	-0.009	-0.009	.	0.006	0.90	8	90	2
5.00	1.875	0.000	-0.010	-0.010	.	0.006	0.89	9	89	2
7.50	1.875	0.000	-0.013	-0.013	.	0.008	0.85	12	85	3
10.00	1.875	0.000	-0.015	-0.015	.	0.011	0.84	12	84	4
1.25	2.500	0.000	-0.011	-0.011	.	0.007	0.90	7	90	3
2.50	2.500	0.000	-0.011	-0.011	.	0.006	0.91	8	91	1
3.75	2.500	0.000	-0.011	-0.011	.	0.006	0.90	8	90	2
5.00	2.500	0.000	-0.011	-0.011	.	0.006	0.89	9	89	2
7.50	2.500	0.000	-0.013	-0.013	.	0.007	0.87	11	87	2
10.00	2.500	0.000	-0.015	-0.015	.	0.010	0.85	11	85	4
1.25	3.750	0.000	-0.018	-0.018	.	0.009	0.87	11	87	2
2.50	3.750	0.000	-0.016	-0.016	.	0.007	0.88	11	88	1
3.75	3.750	0.000	-0.015	-0.015	.	0.007	0.88	11	88	1
5.00	3.750	0.000	-0.015	-0.015	.	0.007	0.87	12	87	1
7.50	3.750	0.000	-0.016	-0.016	.	0.008	0.88	11	88	1
10.00	3.750	0.000	-0.017	-0.017	.	0.010	0.84	13	84	3
1.25	5.000	0.000	-0.026	-0.026	.	0.013	0.86	13	86	1
2.50	5.000	0.000	-0.023	-0.023	.	0.010	0.87	12	87	1
3.75	5.000	0.000	-0.022	-0.022	.	0.009	0.87	12	87	1
5.00	5.000	0.000	-0.021	-0.021	.	0.009	0.84	14	84	2
7.50	5.000	0.000	-0.021	-0.021	.	0.010	0.84	15	84	1
10.00	5.000	0.000	-0.021	-0.021	.	0.011	0.84	13	84	3

Table B.16 shows the detailed results from the evaluation of f_{123}, based on the final model. The true value of the function has some positive values and some negative values, reflecting the mix of synergism and antagonism. The sign of the value of the function was correctly estimated by most simulation runs, and the correct relationship (synergism or antagonism) was identified in many cases. In some cases the magnitude of the relationship did not reach statistical significance, so some cases of true synergism or true antagonism were identified as additive.

Table B.16: Evaluation of f_{123} at all doses, for final model of Scenario 2.

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
2.5	1.25	0.625	1.160	1.185	0.026	0.022	0.030	0.92	0	0	100
5.0	1.25	0.625	1.039	1.066	0.026	0.025	0.024	0.92	0	0	100
7.5	1.25	0.625	0.954	0.981	0.026	0.028	0.022	0.86	0	0	100
10.0	1.25	0.625	0.886	0.913	0.026	0.030	0.021	0.86	0	0	100
15.0	1.25	0.625	0.779	0.804	0.025	0.033	0.021	0.85	0	0	100
20.0	1.25	0.625	0.692	0.717	0.024	0.035	0.029	0.85	0	1	99
2.5	2.50	0.625	1.039	1.059	0.020	0.019	0.021	0.94	0	0	100
5.0	2.50	0.625	0.888	0.908	0.021	0.024	0.016	0.91	0	0	100
7.5	2.50	0.625	0.781	0.802	0.021	0.027	0.015	0.88	0	0	100
10.0	2.50	0.625	0.695	0.717	0.021	0.031	0.015	0.89	0	0	100
15.0	2.50	0.625	0.559	0.580	0.021	0.038	0.017	0.90	0	0	100
20.0	2.50	0.625	0.451	0.471	0.020	0.045	0.025	0.87	0	7	93
2.5	3.75	0.625	0.954	0.971	0.017	0.018	0.019	0.91	0	0	100
5.0	3.75	0.625	0.781	0.799	0.018	0.024	0.015	0.87	0	0	100
7.5	3.75	0.625	0.658	0.677	0.019	0.029	0.014	0.90	0	0	100
10.0	3.75	0.625	0.561	0.580	0.019	0.034	0.014	0.86	0	0	100
15.0	3.75	0.625	0.405	0.424	0.019	0.047	0.017	0.88	0	6	94
20.0	3.75	0.625	0.281	0.300	0.019	0.066	0.027	0.86	0	32	68
2.5	5.00	0.625	0.887	0.902	0.016	0.018	0.019	0.92	0	0	100
5.0	5.00	0.625	0.695	0.713	0.017	0.025	0.015	0.91	0	0	100
7.5	5.00	0.625	0.561	0.579	0.018	0.032	0.015	0.91	0	0	100
10.0	5.00	0.625	0.453	0.472	0.018	0.041	0.015	0.89	0	1	99
15.0	5.00	0.625	0.282	0.301	0.019	0.066	0.019	0.89	0	29	71
20.0	5.00	0.625	0.146	0.164	0.018	0.125	0.030	0.86	0	67	33
2.5	7.50	0.625	0.779	0.795	0.016	0.020	0.024	0.88	0	0	100
5.0	7.50	0.625	0.560	0.577	0.018	0.031	0.019	0.91	0	0	100
7.5	7.50	0.625	0.406	0.424	0.019	0.046	0.019	0.91	0	5	95
10.0	7.50	0.625	0.282	0.302	0.019	0.068	0.020	0.88	0	26	74
15.0	7.50	0.625	0.087	0.107	0.020	0.226	0.025	0.85	1	75	24
20.0	7.50	0.625	-0.069	-0.050	0.020	-0.282	0.037	0.86	13	84	3
2.5	10.00	0.625	0.693	0.711	0.018	0.026	0.033	0.85	0	1	99
5.0	10.00	0.625	0.452	0.472	0.020	0.044	0.028	0.85	0	7	93
7.5	10.00	0.625	0.282	0.303	0.021	0.074	0.027	0.87	0	34	66
10.0	10.00	0.625	0.146	0.168	0.022	0.149	0.028	0.85	0	66	34
15.0	10.00	0.625	-0.069	-0.047	0.022	-0.326	0.034	0.84	10	86	4
20.0	10.00	0.625	-0.241	-0.218	0.023	-0.094	0.048	0.85	35	64	1
2.5	1.25	1.250	1.201	1.218	0.017	0.014	0.021	0.89	0	0	100
5.0	1.25	1.250	1.049	1.068	0.018	0.017	0.015	0.96	0	0	100
7.5	1.25	1.250	0.942	0.961	0.018	0.020	0.014	0.92	0	0	100
10.0	1.25	1.250	0.857	0.875	0.019	0.022	0.013	0.86	0	0	100
15.0	1.25	1.250	0.721	0.739	0.018	0.025	0.016	0.90	0	0	100
20.0	1.25	1.250	0.612	0.630	0.017	0.028	0.025	0.89	0	1	99
2.5	2.50	1.250	1.049	1.061	0.012	0.011	0.013	0.95	0	0	100
5.0	2.50	1.250	0.858	0.871	0.013	0.015	0.009	0.94	0	0	100
7.5	2.50	1.250	0.724	0.737	0.014	0.019	0.008	0.92	0	0	100
10.0	2.50	1.250	0.616	0.630	0.014	0.023	0.008	0.94	0	0	100
15.0	2.50	1.250	0.445	0.459	0.014	0.032	0.011	0.93	0	1	99
20.0	2.50	1.250	0.308	0.322	0.014	0.044	0.021	0.92	0	21	79
2.5	3.75	1.250	0.942	0.951	0.009	0.010	0.012	0.96	0	0	100
5.0	3.75	1.250	0.724	0.734	0.011	0.015	0.008	0.92	0	0	100
7.5	3.75	1.250	0.569	0.581	0.012	0.020	0.007	0.92	0	0	100
10.0	3.75	1.250	0.446	0.458	0.012	0.027	0.008	0.90	0	0	100
15.0	3.75	1.250	0.250	0.263	0.013	0.050	0.012	0.90	0	19	81
20.0	3.75	1.250	0.094	0.106	0.012	0.131	0.023	0.89	1	74	25
2.5	5.00	1.250	0.857	0.865	0.008	0.009	0.013	0.91	0	0	100
5.0	5.00	1.250	0.616	0.626	0.010	0.016	0.008	0.92	0	0	100
7.5	5.00	1.250	0.446	0.457	0.011	0.024	0.008	0.88	0	0	100

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Table B.16 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
10.0	5.00	1.250	0.311	0.322	0.012	0.037	0.009	0.90	0	6	94
15.0	5.00	1.250	0.095	0.108	0.012	0.129	0.013	0.88	0	71	29
20.0	5.00	1.250	-0.077	-0.064	0.012	-0.160	0.025	0.87	12	86	2
2.5	7.50	1.250	0.721	0.729	0.008	0.011	0.017	0.93	0	0	100
5.0	7.50	1.250	0.445	0.456	0.010	0.023	0.011	0.93	0	0	100
7.5	7.50	1.250	0.251	0.263	0.012	0.047	0.011	0.90	0	19	81
10.0	7.50	1.250	0.096	0.108	0.013	0.132	0.012	0.88	0	73	27
15.0	7.50	1.250	-0.151	-0.137	0.014	-0.091	0.018	0.90	28	72	0
20.0	7.50	1.250	-0.348	-0.333	0.014	-0.041	0.031	0.87	75	25	0
2.5	10.00	1.250	0.613	0.623	0.010	0.017	0.026	0.85	0	1	99
5.0	10.00	1.250	0.309	0.322	0.013	0.041	0.018	0.91	0	23	77
7.5	10.00	1.250	0.095	0.109	0.014	0.152	0.018	0.90	0	73	27
10.0	10.00	1.250	-0.076	-0.060	0.016	-0.204	0.019	0.88	12	86	2
15.0	10.00	1.250	-0.347	-0.330	0.017	-0.049	0.026	0.90	75	25	0
20.0	10.00	1.250	-0.564	-0.546	0.018	-0.031	0.040	0.88	93	7	0
2.5	1.25	1.875	1.230	1.246	0.015	0.012	0.020	0.90	0	0	100
5.0	1.25	1.875	1.057	1.073	0.017	0.016	0.014	0.91	0	0	100
7.5	1.25	1.875	0.934	0.951	0.017	0.018	0.013	0.91	0	0	100
10.0	1.25	1.875	0.836	0.854	0.017	0.021	0.013	0.89	0	0	100
15.0	1.25	1.875	0.681	0.698	0.017	0.025	0.016	0.88	0	0	100
20.0	1.25	1.875	0.556	0.573	0.017	0.030	0.026	0.87	0	4	96
2.5	2.50	1.875	1.057	1.067	0.010	0.010	0.013	0.92	0	0	100
5.0	2.50	1.875	0.838	0.849	0.012	0.014	0.008	0.95	0	0	100
7.5	2.50	1.875	0.683	0.696	0.013	0.018	0.007	0.92	0	0	100
10.0	2.50	1.875	0.560	0.573	0.013	0.023	0.008	0.90	0	0	100
15.0	2.50	1.875	0.364	0.378	0.014	0.037	0.011	0.90	0	4	96
20.0	2.50	1.875	0.208	0.221	0.013	0.064	0.022	0.89	0	38	62
2.5	3.75	1.875	0.934	0.942	0.008	0.008	0.012	0.92	0	0	100
5.0	3.75	1.875	0.683	0.693	0.010	0.014	0.007	0.95	0	0	100
7.5	3.75	1.875	0.507	0.518	0.011	0.021	0.007	0.92	0	0	100
10.0	3.75	1.875	0.366	0.377	0.011	0.031	0.008	0.91	0	2	98
15.0	3.75	1.875	0.142	0.154	0.012	0.086	0.012	0.89	0	53	47
20.0	3.75	1.875	-0.037	-0.025	0.012	-0.331	0.024	0.87	7	88	5
2.5	5.00	1.875	0.836	0.843	0.007	0.008	0.013	0.90	0	0	100
5.0	5.00	1.875	0.560	0.569	0.009	0.016	0.008	0.95	0	0	100
7.5	5.00	1.875	0.366	0.376	0.010	0.028	0.007	0.91	0	1	99
10.0	5.00	1.875	0.211	0.222	0.011	0.053	0.008	0.88	0	22	78
15.0	5.00	1.875	-0.036	-0.024	0.012	-0.339	0.014	0.88	8	88	4
20.0	5.00	1.875	-0.233	-0.220	0.012	-0.053	0.026	0.85	56	44	0
2.5	7.50	1.875	0.681	0.688	0.007	0.010	0.017	0.91	0	0	100
5.0	7.50	1.875	0.365	0.374	0.010	0.026	0.010	0.94	0	3	97
7.5	7.50	1.875	0.142	0.154	0.011	0.079	0.010	0.89	0	55	45
10.0	7.50	1.875	-0.035	-0.023	0.012	-0.351	0.011	0.91	6	91	3
15.0	7.50	1.875	-0.317	-0.304	0.014	-0.044	0.018	0.91	79	21	0
20.0	7.50	1.875	-0.543	-0.528	0.015	-0.027	0.032	0.85	94	6	0
2.5	10.00	1.875	0.557	0.566	0.009	0.017	0.025	0.88	0	2	98
5.0	10.00	1.875	0.209	0.221	0.012	0.059	0.017	0.89	0	42	58
7.5	10.00	1.875	-0.036	-0.022	0.014	-0.387	0.016	0.88	7	88	5
10.0	10.00	1.875	-0.232	-0.216	0.015	-0.067	0.017	0.90	58	42	0
15.0	10.00	1.875	-0.542	-0.525	0.017	-0.032	0.025	0.91	98	2	0
20.0	10.00	1.875	-0.790	-0.772	0.018	-0.023	0.041	0.86	99	1	0
2.5	1.25	2.500	1.254	1.271	0.017	0.013	0.020	0.89	0	0	100
5.0	1.25	2.500	1.062	1.081	0.018	0.017	0.015	0.90	0	0	100
7.5	1.25	2.500	0.927	0.946	0.019	0.021	0.013	0.93	0	0	100
10.0	1.25	2.500	0.820	0.839	0.019	0.024	0.013	0.87	0	0	100
15.0	1.25	2.500	0.648	0.668	0.019	0.030	0.016	0.88	0	0	100
20.0	1.25	2.500	0.512	0.531	0.019	0.037	0.027	0.84	0	7	93
2.5	2.50	2.500	1.062	1.074	0.012	0.011	0.014	0.91	0	0	100

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Table B.16 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
5.0	2.50	2.500	0.821	0.835	0.014	0.017	0.009	0.92	0	0	100
7.5	2.50	2.500	0.651	0.666	0.015	0.023	0.008	0.91	0	0	100
10.0	2.50	2.500	0.516	0.531	0.015	0.030	0.008	0.88	0	0	100
15.0	2.50	2.500	0.300	0.316	0.016	0.053	0.012	0.87	0	10	90
20.0	2.50	2.500	0.128	0.144	0.016	0.125	0.023	0.83	1	65	34
2.5	3.75	2.500	0.927	0.937	0.010	0.010	0.013	0.91	0	0	100
5.0	3.75	2.500	0.652	0.663	0.012	0.018	0.008	0.95	0	0	100
7.5	3.75	2.500	0.457	0.470	0.013	0.028	0.007	0.92	0	0	100
10.0	3.75	2.500	0.302	0.316	0.014	0.046	0.008	0.90	0	4	96
15.0	3.75	2.500	0.055	0.070	0.015	0.269	0.013	0.85	2	73	25
20.0	3.75	2.500	-0.142	-0.126	0.015	-0.107	0.025	0.86	25	75	0
2.5	5.00	2.500	0.820	0.828	0.009	0.011	0.014	0.89	0	0	100
5.0	5.00	2.500	0.516	0.527	0.011	0.021	0.009	0.95	0	0	100
7.5	5.00	2.500	0.302	0.315	0.013	0.042	0.008	0.92	0	5	95
10.0	5.00	2.500	0.131	0.145	0.014	0.104	0.009	0.89	0	51	49
15.0	5.00	2.500	-0.140	-0.125	0.015	-0.107	0.015	0.85	30	70	0
20.0	5.00	2.500	-0.357	-0.341	0.016	-0.043	0.028	0.83	80	20	0
2.5	7.50	2.500	0.649	0.658	0.009	0.014	0.018	0.88	0	0	100
5.0	7.50	2.500	0.301	0.313	0.012	0.040	0.011	0.93	0	9	91
7.5	7.50	2.500	0.056	0.070	0.014	0.247	0.010	0.90	1	78	21
10.0	7.50	2.500	-0.140	-0.125	0.015	-0.108	0.012	0.91	27	73	0
15.0	7.50	2.500	-0.450	-0.433	0.017	-0.038	0.019	0.90	97	3	0
20.0	7.50	2.500	-0.698	-0.680	0.018	-0.026	0.034	0.85	100	0	0
2.5	10.00	2.500	0.512	0.524	0.012	0.023	0.026	0.88	0	4	96
5.0	10.00	2.500	0.129	0.143	0.015	0.114	0.017	0.90	0	62	38
7.5	10.00	2.500	-0.141	-0.124	0.017	-0.119	0.016	0.89	28	72	0
10.0	10.00	2.500	-0.356	-0.338	0.018	-0.051	0.017	0.89	81	19	0
15.0	10.00	2.500	-0.698	-0.677	0.020	-0.029	0.026	0.90	100	0	0
20.0	10.00	2.500	-0.971	-0.949	0.022	-0.022	0.042	0.86	100	0	0
2.5	1.25	3.750	1.291	1.316	0.026	0.020	0.025	0.88	0	0	100
5.0	1.25	3.750	1.071	1.099	0.027	0.025	0.018	0.88	0	0	100
7.5	1.25	3.750	0.917	0.945	0.028	0.031	0.016	0.89	0	0	100
10.0	1.25	3.750	0.793	0.822	0.029	0.036	0.016	0.87	0	0	100
15.0	1.25	3.750	0.597	0.627	0.029	0.049	0.019	0.87	0	0	100
20.0	1.25	3.750	0.441	0.470	0.029	0.066	0.029	0.85	0	10	90
2.5	2.50	3.750	1.071	1.092	0.021	0.019	0.019	0.87	0	0	100
5.0	2.50	3.750	0.795	0.818	0.023	0.029	0.012	0.86	0	0	100
7.5	2.50	3.750	0.601	0.625	0.024	0.040	0.011	0.84	0	0	100
10.0	2.50	3.750	0.446	0.471	0.025	0.056	0.011	0.86	0	0	100
15.0	2.50	3.750	0.199	0.225	0.026	0.131	0.015	0.88	0	36	64
20.0	2.50	3.750	0.002	0.028	0.026	16.243	0.026	0.81	7	81	12
2.5	3.75	3.750	0.917	0.935	0.019	0.020	0.019	0.85	0	0	100
5.0	3.75	3.750	0.601	0.622	0.021	0.035	0.012	0.86	0	0	100
7.5	3.75	3.750	0.378	0.401	0.023	0.060	0.010	0.87	0	2	98
10.0	3.75	3.750	0.201	0.224	0.024	0.119	0.011	0.85	0	26	74
15.0	3.75	3.750	-0.082	-0.056	0.025	-0.309	0.016	0.85	10	86	4
20.0	3.75	3.750	-0.307	-0.281	0.026	-0.084	0.028	0.78	67	33	0
2.5	5.00	3.750	0.793	0.811	0.018	0.022	0.019	0.82	0	0	100
5.0	5.00	3.750	0.446	0.466	0.021	0.046	0.012	0.90	0	1	99
7.5	5.00	3.750	0.201	0.223	0.022	0.111	0.011	0.86	0	24	76
10.0	5.00	3.750	0.005	0.029	0.024	4.579	0.012	0.85	3	86	11
15.0	5.00	3.750	-0.305	-0.280	0.025	-0.083	0.018	0.85	76	24	0
20.0	5.00	3.750	-0.554	-0.527	0.026	-0.048	0.031	0.83	96	4	0
2.5	7.50	3.750	0.598	0.616	0.019	0.031	0.023	0.83	0	1	99
5.0	7.50	3.750	0.199	0.221	0.022	0.109	0.014	0.89	0	38	62
7.5	7.50	3.750	-0.081	-0.057	0.024	-0.294	0.013	0.85	9	89	2
10.0	7.50	3.750	-0.305	-0.280	0.026	-0.084	0.014	0.88	79	21	0
15.0	7.50	3.750	-0.660	-0.633	0.028	-0.042	0.022	0.87	100	0	0

Continued on Next Page

Table B.16 – Continued

DrugA	DrugB	DrugC	true	ave	abs.bias	rel.bias	mse	cr.ci	p.ant	p.add	p.syn
20.0	7.50	3.750	-0.944	-0.915	0.029	-0.031	0.038	0.86	100	0	0
2.5	10.00	3.750	0.441	0.462	0.021	0.048	0.030	0.82	0	10	90
5.0	10.00	3.750	0.002	0.027	0.025	10.807	0.019	0.87	3	87	10
7.5	10.00	3.750	-0.306	-0.279	0.027	-0.088	0.018	0.85	72	28	0
10.0	10.00	3.750	-0.553	-0.524	0.029	-0.052	0.019	0.86	100	0	0
15.0	10.00	3.750	-0.944	-0.912	0.032	-0.033	0.029	0.85	100	0	0
20.0	10.00	3.750	-1.256	-1.223	0.033	-0.027	0.047	0.85	100	0	0
2.5	1.25	5.000	1.320	1.358	0.038	0.029	0.036	0.81	0	0	100
5.0	1.25	5.000	1.079	1.119	0.040	0.037	0.028	0.85	0	0	100
7.5	1.25	5.000	0.909	0.950	0.041	0.046	0.025	0.86	0	0	100
10.0	1.25	5.000	0.773	0.815	0.042	0.055	0.024	0.88	0	0	100
15.0	1.25	5.000	0.557	0.600	0.043	0.077	0.026	0.87	0	2	98
20.0	1.25	5.000	0.384	0.427	0.043	0.111	0.036	0.85	0	17	83
2.5	2.50	5.000	1.079	1.112	0.034	0.031	0.030	0.81	0	0	100
5.0	2.50	5.000	0.775	0.811	0.036	0.047	0.022	0.86	0	0	100
7.5	2.50	5.000	0.561	0.598	0.038	0.067	0.019	0.86	0	1	99
10.0	2.50	5.000	0.390	0.428	0.039	0.099	0.019	0.86	0	7	93
15.0	2.50	5.000	0.118	0.158	0.040	0.340	0.022	0.87	0	57	43
20.0	2.50	5.000	-0.099	-0.058	0.041	-0.410	0.033	0.84	12	82	6
2.5	3.75	5.000	0.908	0.940	0.032	0.035	0.030	0.81	0	0	100
5.0	3.75	5.000	0.561	0.595	0.035	0.062	0.021	0.85	0	1	99
7.5	3.75	5.000	0.316	0.352	0.036	0.115	0.019	0.84	0	8	92
10.0	3.75	5.000	0.120	0.158	0.038	0.314	0.019	0.84	0	54	46
15.0	3.75	5.000	-0.191	-0.151	0.039	-0.207	0.023	0.85	33	65	2
20.0	3.75	5.000	-0.439	-0.399	0.040	-0.092	0.036	0.81	86	14	0
2.5	5.00	5.000	0.773	0.804	0.031	0.040	0.030	0.80	0	1	99
5.0	5.00	5.000	0.390	0.424	0.034	0.088	0.021	0.86	0	4	96
7.5	5.00	5.000	0.120	0.156	0.036	0.302	0.019	0.86	1	54	45
10.0	5.00	5.000	-0.095	-0.058	0.038	-0.396	0.020	0.84	13	83	4
15.0	5.00	5.000	-0.437	-0.397	0.040	-0.091	0.026	0.85	91	9	0
20.0	5.00	5.000	-0.710	-0.669	0.041	-0.058	0.040	0.81	98	2	0
2.5	7.50	5.000	0.557	0.589	0.032	0.057	0.033	0.81	0	2	98
5.0	7.50	5.000	0.118	0.154	0.036	0.301	0.023	0.85	1	61	38
7.5	7.50	5.000	-0.190	-0.153	0.038	-0.199	0.021	0.85	36	63	1
10.0	7.50	5.000	-0.437	-0.397	0.040	-0.091	0.022	0.84	90	10	0
15.0	7.50	5.000	-0.828	-0.786	0.042	-0.051	0.031	0.86	100	0	0
20.0	7.50	5.000	-1.140	-1.096	0.044	-0.039	0.048	0.86	100	0	0
2.5	10.00	5.000	0.385	0.419	0.035	0.090	0.040	0.79	0	18	82
5.0	10.00	5.000	-0.099	-0.060	0.039	-0.392	0.027	0.83	12	83	5
7.5	10.00	5.000	-0.438	-0.397	0.041	-0.094	0.025	0.85	87	13	0
10.0	10.00	5.000	-0.710	-0.666	0.043	-0.061	0.027	0.84	99	1	0
15.0	10.00	5.000	-1.140	-1.094	0.046	-0.041	0.038	0.86	100	0	0
20.0	10.00	5.000	-1.484	-1.435	0.049	-0.033	0.058	0.85	100	0	0

Appendix C

Biased Bootstrap Estimates in the Kong and Lee Semiparametric Variance Estimation

When the Kong and Lee semiparametric method was initially extended to handle 3 drugs, the variance and confidence intervals of the nonparametric part were estimated using a wild bootstrap in a manner similar to that originally used by Kong and Lee[26]. But some preliminary evaluation of the confidence intervals revealed a possible problem in the bootstrap estimates used to construct the confidence intervals. Further investigation suggested that the problem was not limited to the extended Kong and Lee confidence intervals, and that it was also present in the confidence intervals in the original Kong and Lee method.

The variance estimation method described in the Section 2.5.4 was implemented in a program that analyzed a case study in the original Kong and Lee paper [20]. Although the estimates of the confidence interval appear reasonable, the implementation of the method seems to have a problem with the estimates of of $f^*(d_{1i}, d_{2i})$. For the data analyzed in the original case study, Figure C.1 shows the estimates of $\hat{f}(d_{1i}, d_{2i})$ as a solid black lines at a number of dose levels. The estimates of $f^*(d_{1i}, d_{2i})$ from 19 bootstrapped runs are shown as dashed lines. As the figure shows, the estimates of $f^*(d_{1i}, d_{2i})$ are generally overestimating $\hat{f}(d_{1i}, d_{2i})$, and thus underestimating the magnitude of the synergy. Because the estimates of $f^*(d_{1i}, d_{2i})$ are not centered around $\hat{f}(d_{1i}, d_{2i})$, the sample standard deviation used to estimate the variance is probably overestimating the variance of $\hat{f}(d_{1i}, d_{2i})$.

The source of this problem has not yet been determined, but it seems to be related to the wild bootstrap and the two-step nature of the Kong and Lee method, first estimating a parametric additive model, and then estimating a nonparametric model

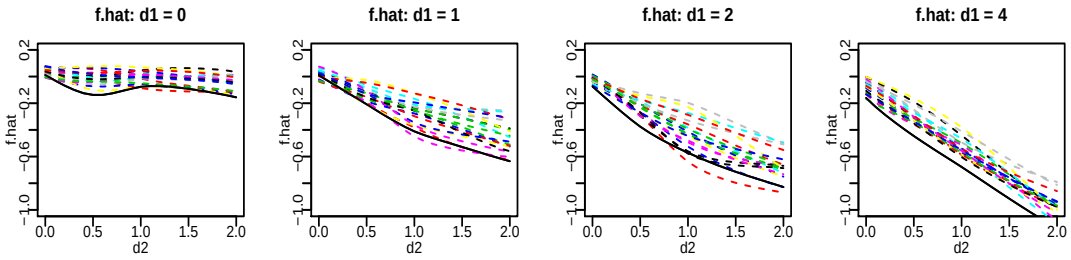

Figure C.1: Original estimated fit and bootstrapped fits.

of the departure from additivity.

The Kong and Lee variance estimation method is based on the methods of Härdle and Marron [15], and Davison and Hinkley [8]. Although bootstrapping of linear regression is usually done based on the residuals, Härdle and Marron identify the problem of bootstrapping residuals from nonparametric regression, and proposed to instead use a wild bootstrap with each original residual multiplied by a random component and added back to its original fitted value to create a new generated observation.

One complication of the Kong and Lee method comes from the two-step nature of the method. The observations that are fitted in the nonparametric regression of the second step of the method are not the original observations, they are actually residuals from the parametric regression fit of the additive model that was fitted in the first step of the method. So there are two possibilities to consider as the "original" observations in bootstrapping the semiparametric model: the actual original observations, or the observations that were originally fitted by the nonparametric regression, which were really residuals from the first step of the method. The Kong and Lee method uses the actual original observations.

Another complication of the Kong and Lee method seems to be related to the special nature of some of the original observations. The original observations from treatments where only one of the drugs is present are used to estimate the dose-response curve for that drug alone. Those same observations are also used to estimate the additive model surface used in the first step of the method.

There seems to be a problem with generating wild bootstrap samples of these observations where only one drug is present, although the exact nature of the problem is not yet clear. One of the components of the wild bootstrap samples generated in is an Step 4 is oversmoothed estimate of $f(d_{1i}, d_{2i})$. But this estimate may not improve the parametric fit very much, because by definition $f(d_1, d_2)$ should be 0 when only one drug is present, so perhaps this is causing a problem when it is used to estimate the additive surface. Or perhaps the problem is that the Härdle and Marron results are valid for generating bootstrap samples to make estimates of nonparametric regressions, but the Kong and Lee method is using the generated bootstrap samples first in a parametric regression, and then using the residuals from that regression in a nonparametric regression. And perhaps the generated bootstrap samples estimate a very different additive model than the original samples did. Additional work is necessary to identify the cause of the problem.

One possible solution to the problem has been investigated and seems to show some promise. Step 4 of the variance estimation algorithm was modified to treat observations where only one drug is present differently than observations where both drugs are present. The existing algorithm was used for observations where both drugs are present, but for observations where only one drug was present, the original observation was used, rather than a generated observation. This ensures that the same parametric additive model will be fit to each set of bootstrap samples. This also effectively modifies the algorithm to treat the residuals from the parametric model as the "original observations", rather than the original observations themselves. In some sense that interpretation may more closely match the assumptions of Härdle and Marron, and Davison and Hinkley, because the residuals from the parametric model really were the observations originally fit by the nonparametric regression.

Figure C.2 shows the results when the variance estimation algorithm is modified as described in the previous paragraph. As before, the estimates of $\hat{f}(d_{1i}, d_{2i})$ are shown as solid black lines, and the estimates of $f^*(d_1, d_2)$ from 19 bootstrapped runs are shown as dashed lines. But now the estimates of $f^*(d_{1i}, d_{2i})$ are more evenly clustered around $\hat{f}(d_{1i}, d_{2i})$, rather than being clustered above it as in Figure C.1.

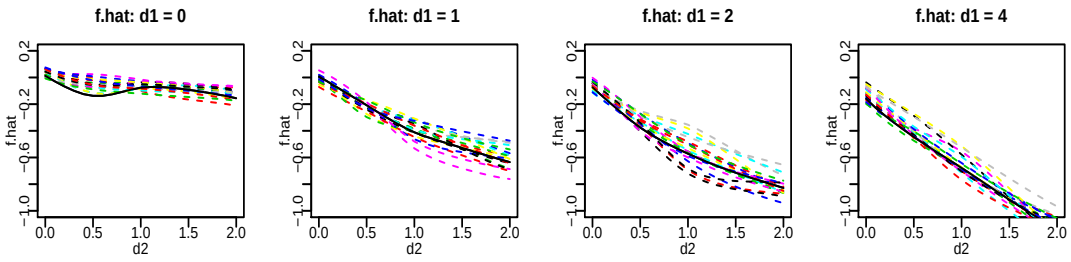

Figure C.2: Revised estimated fit and bootstrapped fits.

Appendix D

Extended Kong and Lee Semiparametric Method Model Function Goodness of Fit Tables

In Section 6.6, the extended Kong and Lee semiparametric method evaluated the performance of the method by evaluating the estimation of the model functions, f_{12}, f_{13}, f_{23} and f_{123} at each dose combination. Because of the large number of dose combinations, particularly for the f_{123} function, the results of the evaluation were summarized graphically in that section. The following tables show the individual results at each dose combination.

Table D.1 shows the performance for estimating function f_{12}, which models any two-way synergy between drugs 1 and 2. The interpretation of the columns is the same as the interpretation of the columns of Table D.3, although here the reported doses are for drugs 1 and 2, and the results are for f_{12} instead of f_{23}.

The true value of f_{12} ("true") is 0 at all dose combinations, and in most cases the average estimates ("ave") were reasonably close, as shown by the relatively small bias ("abs.bias"). The variance estimates of f_{12} ("var") seem fairly good, with the proportion of estimated confidence intervals containing the true value of f_{12} being close to 0.95 in most cases. f_{12} is additive at all dose combinations, and identified as such at least 85% of the time for all dose combinations except, where it was only correctly identified 80% of the time.

Table D.2 shows the performance for estimating function f_{13}, which models any two-way synergy between drugs 1 and 3. The interpretation of the columns is the same as the interpretation of the columns of Table D.3, although here the reported doses are for drugs 1 and 3, and the results are for f_{13} instead of f_{23}.

The true value of f_{13} ("true") is 0 at all dose combinations, and in most cases

Table D.1: Simulation results for f_{12}

dose1	dose2	true	ave	abs.bias	pct.bias	var	cr.bci	p.ant	p.add	p.syn
2.5	1.25	0.0	-0.005	-0.005	NA	0.002	100	0	100	0
5.0	1.25	0.0	0.005	0.005	NA	0.002	99	0	99	1
10.0	1.25	0.0	0.002	0.002	NA	0.002	95	2	95	3
20.0	1.25	0.0	-0.004	-0.004	NA	0.002	85	4	85	11
2.5	2.50	0.0	-0.003	-0.003	NA	0.003	100	0	100	0
5.0	2.50	0.0	0.005	0.005	NA	0.002	99	1	99	0
10.0	2.50	0.0	0.011	0.011	NA	0.003	93	4	93	3
20.0	2.50	0.0	-0.006	-0.006	NA	0.003	83	4	83	13
2.5	5.00	0.0	-0.002	-0.002	NA	0.003	98	2	98	0
5.0	5.00	0.0	0.009	0.009	NA	0.003	96	3	96	1
10.0	5.00	0.0	-0.002	-0.002	NA	0.004	91	6	91	3
20.0	5.00	0.0	-0.006	-0.006	NA	0.003	88	2	88	10
2.5	10.00	0.0	-0.005	-0.005	NA	0.003	93	1	93	6
5.0	10.00	0.0	-0.006	-0.006	NA	0.004	85	9	85	6
10.0	10.00	0.0	0.005	0.005	NA	0.004	87	7	87	6
20.0	10.00	0.0	-0.002	-0.002	NA	0.006	86	6	86	8

the average estimates ("ave") were reasonably close, as shown by the relatively small bias ("abs.bias"). The variance estimates of f_{13} ("var") seem fairly good, with the proportion of estimated confidence intervals containing the true value of f_{13} being close to 0.95 in most cases. f_{13} is additive at all dose combinations, and was generally identified as such, except at the highest dose of drug 1, where f_{13} was misidentified as either antagonistic or synergistic up to 35% of the time.

Table D.2: Simulation results for f_{13}

dose1	dose3	true	ave	abs.bias	pct.bias	var	cr.bci	p.ant	p.add	p.syn
2.5	0.625	0.0	-0.001	-0.001	NA	0.002	98	0	98	2
5.0	0.625	0.0	0.002	0.002	NA	0.002	98	1	98	1
10.0	0.625	0.0	0.004	0.004	NA	0.002	97	2	97	1
20.0	0.625	0.0	0.005	0.005	NA	0.002	79	12	79	9
2.5	1.250	0.0	-0.005	-0.005	NA	0.002	97	0	97	3
5.0	1.250	0.0	0.003	0.003	NA	0.002	96	2	96	2
10.0	1.250	0.0	0.007	0.007	NA	0.002	93	4	93	3
20.0	1.250	0.0	0.010	0.010	NA	0.003	79	13	79	8
2.5	2.500	0.0	-0.008	-0.008	NA	0.003	91	5	91	4
5.0	2.500	0.0	-0.002	-0.002	NA	0.004	89	8	89	3
10.0	2.500	0.0	0.012	0.012	NA	0.003	83	11	83	6
20.0	2.500	0.0	0.016	0.016	NA	0.003	82	14	82	4
2.5	5.000	0.0	0.000	0.000	NA	0.003	82	11	82	7
5.0	5.000	0.0	0.004	0.004	NA	0.004	82	9	82	9
10.0	5.000	0.0	0.019	0.019	NA	0.004	81	16	81	3
20.0	5.000	0.0	0.019	0.019	NA	0.005	78	16	78	6

Table D.3 shows the performance for estimating function f_{23}, which models any two-way synergy between drugs 2 and 3. Each row of the table describes the performance of the method for a particular dose combination of the two drugs, indicated by the columns "dose2" and "dose3". "true" is the true value of the function f_{23} at that dose combination. 'Each simulation run estimated the value of f_{23} at that dose combination, and 'ave" is the average of of those estimated values. "abs.bias" is the mean bias of the estimated values of f_{23}, that is the mean of the bias for each estimated f_{23}. Each simulation run also estimated the variance of its f_{23} estimate, and the mean of the variance estimates is reported as "var". The results of each simulation run's estimate of f_{23} and the variance of the estimate were used to construct a 95% confidence interval for f_{23}; "cr.bci" reports the proportion of those confidence intervals that contained the true value of f_{23}. The confidence interval from each simulation run was used to determine if that drug combination was antagonistic, additive, or synergistic; "p.ant" reports the percentage of runs which identified the combination as antagonistic, "p.add" reports the percentage of runs which identified the combination as additive, and "p.syn" reports the percentage of runs which identified the combination as synergistic.

In most cases the average estimated value of f_{23} ("ave") was reasonably close to its true value ("true"), as shown by the relatively small mean bias ("abs.bias"). The variance estimates ("var") may be underestimating the variance because the proportion of confidence intervals including the true value of f_{23} ("cr.bci") did not reach the specified confidence level of 0.95. The true function f_{23} was synergistic at all dose combinations, and except for two low dose combinations, f_{23} was identified as significantly synergistic in at least 85% of the simulation runs.

Table D.4 shows the performance for estimating function f_{123}, which models any three-way synergy between all three drugs. The interpretation of the columns is the same as the interpretation of the columns of Table D.3, although here the reported doses are for drugs 1, 2 and 3, and the results are for f_{123} instead of f_{23}.

In most cases the average estimated value of f_{123} ("ave") was reasonably close to its true value ("true"), as shown by the relatively small mean bias ("abs.bias"). The variance estimates ("var") seem to be fairly good with the proportion of estimated

Table D.3: Simulation results for f_{23}

dose2	dose3	true	ave	abs.bias	pct.bias	var	cr.bci	p.ant	p.add	p.syn
1.25	0.625	-0.125	-0.092	0.034	-27.1	0.003	71	0	43	57
2.50	0.625	-0.156	-0.117	0.039	-25.3	0.002	69	0	17	83
5.00	0.625	-0.187	-0.136	0.050	-27.0	0.002	53	0	9	91
10.00	0.625	-0.216	-0.172	0.044	-20.2	0.002	58	0	2	98
1.25	1.250	-0.162	-0.138	0.025	-15.1	0.003	81	0	15	85
2.50	1.250	-0.202	-0.176	0.026	-12.7	0.003	84	0	6	94
5.00	1.250	-0.241	-0.212	0.029	-12.1	0.003	80	0	4	96
10.00	1.250	-0.279	-0.259	0.020	-7.2	0.003	71	0	2	98
1.25	2.500	-0.202	-0.180	0.021	-10.6	0.003	80	0	12	88
2.50	2.500	-0.251	-0.240	0.011	-4.3	0.003	83	0	4	96
5.00	2.500	-0.300	-0.297	0.002	-0.8	0.003	85	0	0	100
10.00	2.500	-0.347	-0.339	0.008	-2.3	0.004	77	0	2	98
1.25	5.000	-0.241	-0.196	0.045	-18.7	0.004	61	0	17	83
2.50	5.000	-0.300	-0.279	0.020	-6.7	0.004	69	0	4	96
5.00	5.000	-0.358	-0.353	0.006	-1.5	0.004	74	0	3	97
10.00	5.000	-0.415	-0.413	0.002	-0.4	0.005	80	0	1	99

confidence intervals containing the true value of f_{123} ("cr.bci") being close to 0.95 in most cases. The true function f_{123} is synergistic at low doses, and antagonistic at high doses of drug 1, around 20. At low doses of drug 1, the synergism was correctly identified in most cases ("p.syn"). For the high dose of drug 1, the antagonism was sometimes significant at low doses of drug 2 and drug 3 ("p.ant" in Table D.4), and was significant for the majority of cases with high doses of drugs 2 and 3 ("p.ant" in Table D.4).

Table D.4: Simulation results for f_{123}

dose1	dose2	dose3	true.f0	f0.ave	abs.bias	pct.bias	f0.var	cr.bci	p.ant	p.add	p.syn
2.5	1.25	0.625	-0.158	-0.130	0.028	-18.0	0.004	93	0	37	63
5.0	1.25	0.625	-0.178	-0.148	0.030	-17.0	0.004	92	0	30	70
10.0	1.25	0.625	-0.196	-0.162	0.034	-17.5	0.004	93	0	24	76
20.0	1.25	0.625	0.170	0.098	-0.072	-42.2	0.005	86	26	74	0
2.5	2.50	0.625	-0.183	-0.166	0.017	-9.3	0.004	93	0	24	76
5.0	2.50	0.625	-0.206	-0.190	0.016	-7.9	0.005	96	0	11	89
10.0	2.50	0.625	-0.227	-0.215	0.012	-5.2	0.005	96	0	9	91
20.0	2.50	0.625	0.196	0.133	-0.064	-32.3	0.005	82	53	47	0
2.5	5.00	0.625	-0.206	-0.175	0.030	-14.8	0.005	95	0	24	76
5.0	5.00	0.625	-0.232	-0.201	0.031	-13.3	0.005	91	0	17	83
10.0	5.00	0.625	-0.256	-0.216	0.039	-15.3	0.006	90	0	16	84
20.0	5.00	0.625	0.221	0.137	-0.085	-38.2	0.006	74	49	51	0
2.5	10.00	0.625	-0.227	-0.186	0.041	-17.9	0.005	87	0	29	71
5.0	10.00	0.625	-0.256	-0.214	0.042	-16.4	0.006	88	0	18	82
10.0	10.00	0.625	-0.282	-0.244	0.037	-13.3	0.007	94	0	10	90
20.0	10.00	0.625	0.244	0.157	-0.087	-35.5	0.008	74	55	45	0
2.5	1.25	1.250	-0.187	-0.179	0.009	-4.6	0.006	96	0	28	72
5.0	1.25	1.250	-0.211	-0.206	0.005	-2.4	0.006	94	0	17	83
10.0	1.25	1.250	-0.233	-0.226	0.007	-2.9	0.005	94	0	11	89
20.0	1.25	1.250	0.202	0.141	-0.061	-30.0	0.006	91	45	55	0
2.5	2.50	1.250	-0.217	-0.231	-0.014	6.5	0.006	94	0	15	85

Continued on Next Page

Table D.4 – Continued

dose1	dose2	dose3	true.f0	f0.ave	abs.bias	pct.bias	f0.var	cr.bci	p.ant	p.add	p.syn
5.0	2.50	1.250	-0.244	-0.270	-0.026	10.5	0.007	93	0	6	94
10.0	2.50	1.250	-0.269	-0.303	-0.033	12.4	0.006	90	0	5	95
20.0	2.50	1.250	0.233	0.199	-0.034	-14.7	0.007	95	68	32	0
2.5	5.00	1.250	-0.244	-0.242	0.003	-1.0	0.007	96	0	16	84
5.0	5.00	1.250	-0.275	-0.290	-0.015	5.6	0.008	94	0	9	91
10.0	5.00	1.250	-0.303	-0.310	-0.007	2.1	0.008	96	0	8	92
20.0	5.00	1.250	0.263	0.213	-0.050	-18.9	0.008	87	66	34	0
2.5	10.00	1.250	-0.269	-0.255	0.014	-5.2	0.008	93	0	22	78
5.0	10.00	1.250	-0.303	-0.300	0.003	-1.1	0.009	94	0	14	86
10.0	10.00	1.250	-0.334	-0.337	-0.003	0.9	0.009	95	0	8	92
20.0	10.00	1.250	0.289	0.233	-0.057	-19.6	0.012	82	60	40	0
2.5	1.25	2.500	-0.217	-0.208	0.008	-3.8	0.007	95	0	27	73
5.0	1.25	2.500	-0.244	-0.242	0.002	-1.0	0.007	93	0	15	85
10.0	1.25	2.500	-0.269	-0.269	-0.000	0.1	0.007	94	0	11	89
20.0	1.25	2.500	0.233	0.179	-0.054	-23.1	0.007	92	54	46	0
2.5	2.50	2.500	-0.251	-0.275	-0.024	9.7	0.008	85	0	15	85
5.0	2.50	2.500	-0.282	-0.325	-0.043	15.2	0.008	88	0	9	91
10.0	2.50	2.500	-0.311	-0.362	-0.051	16.5	0.008	86	0	2	98
20.0	2.50	2.500	0.269	0.263	-0.006	-2.3	0.009	94	80	20	0
2.5	5.00	2.500	-0.282	-0.286	-0.004	1.3	0.009	92	0	14	86
5.0	5.00	2.500	-0.318	-0.340	-0.022	7.0	0.009	92	0	10	90
10.0	5.00	2.500	-0.351	-0.375	-0.024	6.9	0.009	94	0	5	95
20.0	5.00	2.500	0.304	0.298	-0.006	-2.0	0.010	92	85	15	0
2.5	10.00	2.500	-0.311	-0.305	0.006	-2.0	0.010	94	0	16	84
5.0	10.00	2.500	-0.351	-0.369	-0.019	5.4	0.012	90	0	15	85
10.0	10.00	2.500	-0.386	-0.422	-0.036	9.3	0.011	89	0	6	94
20.0	10.00	2.500	0.335	0.320	-0.014	-4.3	0.014	86	72	28	0
2.5	1.25	5.000	-0.244	-0.227	0.017	-7.1	0.008	90	0	23	77
5.0	1.25	5.000	-0.275	-0.262	0.013	-4.7	0.009	90	0	22	78
10.0	1.25	5.000	-0.303	-0.291	0.012	-4.0	0.009	88	0	15	85
20.0	1.25	5.000	0.263	0.179	-0.084	-31.9	0.009	78	50	50	0
2.5	2.50	5.000	-0.282	-0.291	-0.008	2.9	0.011	91	0	21	79
5.0	2.50	5.000	-0.318	-0.354	-0.036	11.2	0.010	83	0	10	90
10.0	2.50	5.000	-0.351	-0.387	-0.036	10.3	0.011	86	0	11	89
20.0	2.50	5.000	0.304	0.277	-0.027	-8.9	0.013	90	75	25	0
2.5	5.00	5.000	-0.318	-0.315	0.003	-1.0	0.012	87	0	19	81
5.0	5.00	5.000	-0.358	-0.375	-0.016	4.6	0.012	90	0	11	89
10.0	5.00	5.000	-0.395	-0.402	-0.007	1.7	0.014	88	0	11	89
20.0	5.00	5.000	0.342	0.339	-0.003	-0.9	0.014	84	80	20	0
2.5	10.00	5.000	-0.351	-0.308	0.043	-12.2	0.014	89	0	26	74
5.0	10.00	5.000	-0.395	-0.390	0.005	-1.2	0.015	91	0	21	79
10.0	10.00	5.000	-0.435	-0.441	-0.005	1.2	0.016	90	0	8	92
20.0	10.00	5.000	0.377	0.374	-0.003	-0.8	0.019	86	78	22	0

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Vita

John Oleynick

1988	B. S. in Computer Science, Northeastern University, Boston, MA
1998	M. S. in Computer Engineering, Santa Clara University, Santa Clara, CA
2005	M. S. in Statistics, Rutgers University, New Brunswick, NJ
6/88-2/92	Software Engineer, Stratus Computer, Marlboro, MA
2/92-4/95	Systems Programmer, Rutgers University, Piscataway, NJ
4/95-7/96	Software Engineer, Pipeline Associates, Morris Plains, NJ
7/96-2/99	Senior Software Engineer, Centigram Communications, San Jose, CA
2/99-10/02	Senior Member of Technical Staff, DIVA Systems, Princeton, NJ
6/04-11/06	Senior Statistician, Cancer Institute of New Jersey, New Brunswick, NJ
11/06-6/07	Statistician, Merck and Company, Rahway, NJ
6/07-	Senior Biostatistician, Janssen Pharmaceutical R & D, Spring House, PA