DEPENDENT BERKSON ERRORS IN LINEAR AND NONLINEAR MODELS

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Abstract

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Often predictor variables in regression models are measured with errors. This is known as an errors-in-variables (EIV) problem. The statistical analysis of the data ignoring the EIV is called naive analysis. As a result, the variance of the errors is underestimated. This affects any statistical inference that may subsequently be made about the model parameter estimates or the response prediction. In some cases (e.g. quadratic polynomial models) the parameter estimates and the model prediction is biased. The errors can occur in different ways. These errors are mainly classified into classical (i.e. occur in observational studies) or Berkson type (i.e. occur in designed experiments).

This thesis addresses the problem of the Berkson EIV and their effect on the statistical analysis of data fitted using linear and nonlinear models. In particular, the case when the errors are dependent and have heterogeneous variance is studied. Both analytical and empirical tools have been used to develop new approaches for dealing with this type of errors.

Two different scenarios are considered: mixture experiments where the model to be estimated is linear in the parameters and the EIV are correlated; and bioassay dose-response studies where the model to be estimated is nonlinear. EIV following Gaussian distribution, as well as the much less investigated non-Gaussian distribution are examined.

When the errors occur in mixture experiments both analytical and empirical results showed that the naive analysis produces biased and inefficient estimators for the model parameters. The magnitude of the bias depends on the variances of the EIV for the mixture components, the model and its parameters. First and second Scheffé polynomials are used to fit the response. To adjust for the EIV, four different approaches of corrections are proposed. The statistical properties of the estimators are investigated, and compared with the naive analysis estimators. Analytical and empirical weighted regression calibration methods are found to give the most accurate and efficient results. The approaches require the error variance to be known prior to the analysis. The robustness of the adjusted approaches for misspecified variance was also examined.

Different error scenarios of EIV in the settings of concentrations in bioassay dose-response studies are studied (i.e. dependent and independent errors). The scenarios are motivated by real-life examples. Comparisons between the effects of the errors are illustrated using the 4-prameter Hill model. The results show that when the errors are non-Gaussian, the nonlinear least squares approach produces biased and inefficient estimators. An extension of the well-known simulation-extrapolation (SIMEX) method is developed for the case when the EIV lead to biased model parameters estimators, and is called Berkson simulation-extrapolation (BSIMEX). BSIMEX requires the error variance to be known. The robustness of the adjusted approach for misspecified variance is examined. Moreover, it is shown that BSIMEX performs better than the regression calibration methods when the EIV are dependent, while the regression calibration methods are preferable when the EIV are independent.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright

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Publications

- The material in Chapter 3 is based on the paper:

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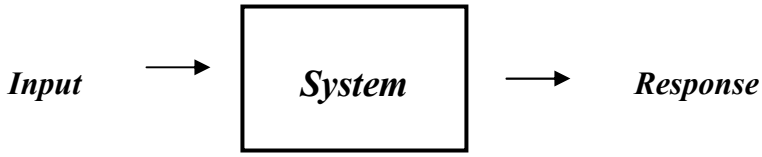
Althubaiti, A., & Donev, A. Mixtures Experiments with Mixing Errors. First version: 12 June 2009. Research Report No. 10, 2009, Probability and Statistics Group, School of Mathematics, The University of Manchester

1 An Overview and Introduction

A statistical regression model can be described as a tool for analyzing and measuring the relationship between two or more variables. There are two types of variables in a regression model, the type whose value is influenced, which are called dependent or response variables, and the type which do the influencing, which are called independent, regressor or predictor variables. The relation between the response and the predictor variables can be described as a system that depends on an input and an output (see for example Figure 1.1), or by $y = f(x)$, where y is the output that depends on the input x.

Figure 1.1 The relation between a response and an input



The problem of measurement errors can occur in both variables. However, most of the regression methods assume that the response is measured with errors and the predictor variable is error free. Therefore, when errors occur in the predictor variable, these methods' assumptions are no longer valid. Usually, studies that investigate such errors are referred to as studies of errors-in-variables (EIV).

When investigating the problem of EIV, the following aspects need to be considered: the possible sources of errors in the study; the error structure; and the procedures to be taken to reduce or eliminate the effects of the errors.

In this work, we are interested in the problem of EIV. We believe that, in practice, it is unlikely for a measurement to be taken without an error, which gives us a strong motivation to study the problem.

The effects of EIV on the analysis of data should not be ignored. These effects could be serious as they generally cause increased variability and bias. The effects are also likely to vary according to the type of statistical response model (e.g. linear or nonlinear in the parameters) used to fit the data.

Another factor which influences these effects is the type of study: observational or designed. In an observational study, the values of some or all of the predictor variables are measured, but are not chosen or set. In a designed study, these values are set by the experimenter in such a way that statistical analysis can be used to draw conclusions on the required point of interest.

Each of these studies, for the most part encounters a specific type of EIV. In observational studies, a classical EIV tends to occur, while, in designed studies, a Berkson error is most likely to occur. However, some observational studies have also examined the assumption that both classical and Berkson errors are present in the data. An example of this is when the true value of a certain individual is estimated with errors, based on observed values from previous knowledge or from other individuals in the study. The estimated value might be the average of several observations (see Küchenhoff et al., 2007). More details about classical and Berkson errors will be given in Chapter 2.

The literature regarding studies of EIV focuses on the case where the EIV are of the classical, independent and homogeneous type. Cases in which the errors have a complicated

structure, such as dependent errors, or when the errors are of the Berkson type, are not sufficiently addressed, maybe as a result of their inherently complicated effects on the results.

Therefore, to distinguish our work from what has previously been presented in the literature, we mainly study the effect of dependent and heterogeneous Berkson errors on the analysis of data in experimental design settings. Moreover, to include a variety of response models in the study, we examine the effects on both linear and nonlinear response models. This will also help us to derive general results and conclusions. Correction approaches for these effects are proposed. The effects and the appropriate correction methods are tested using computer simulation programs written using the R CRAN software package (R, 2000), and run on a computer with an Intel Dual Core processor, 2.13 GHz, 2.00 GB RAM under the Microsoft Windows XP professional operating system.

In general, the novelty of this work lies in the exploration of error structures that have not been examined before. These structures are supported by practical examples to show the possible domains in which they could occur. In addition, new correction approaches for the Berkson EIV are proposed, by modifying some of the well-known techniques used to correct for classical EIV. The layout of the remainder of this dissertation is as follows.

In Chapter 2 we introduce the basic concepts related to both classical and Berkson EIV. These concepts include the possible sources of errors, error structures, and the effects of the errors on the analysis of observational and designed studies. A review of well-known procedures that can be taken to reduce or eliminate the effects of these errors is also presented.

Chapter 3 provides a comprehensive description of the effects of errors on linear response models used to fit data in mixture experiments. It presents novel analytic and empirical results regarding the effects of EIV on mixture experiments. Using the delta method, we show that ignoring the errors in the analysis leads to approximately biased and inefficient estimates of the model parameters. The response models are also found to be approximately

biased and to include heterogeneous variance. These results are verified using simulation examples.

Correction approaches are then proposed in the spirit of the well-known regression calibration method. The new approaches provide a direct and simple way to analyze the data from mixture experiments while taking into account EIV. Moreover, we propose a method to enhance the process of manufacturing mixtures when EIV cannot be avoided, which can be used to increase the quality of the manufactured mixtures.

Chapter 4 studies the effect of EIV on nonlinear models. We choose a model often used in practice to fit the dose-response relation in bioassay studies. To examine the effects of different error structures, we conduct a large number of simulation studies. For example, we investigate the effects of non-Gaussian and Gaussian errors on the analysis of data. We also study the effects of independently distributed EIV. The results show that the severity of the effect of ignoring the errors in the analysis depends on the error assumptions.

When the errors are non-Gaussian, the nonlinear least squares estimators are found to be biased and inefficient. Therefore, we propose a correction approach (BSIMEX) to obtain approximately unbiased and efficient estimates of model parameters. The new approach is a modification of the well-known simulation-extrapolation method. It is shown that BSIMEX performs better than regression calibration approaches when the EIV are dependent, while the latter are preferable when the EIV are independent.

The proposed approaches of analysis of EIV models for dependent Berkson errors in Chapters 3 and 4 require some knowledge about the distributional properties of the error components, which need to be specified prior to the experiment. We also investigate the robustness of the approaches when this knowledge is not accurate.

Chapter 5 presents a summary of the work detailed in Chapters 3 and 4, and finishes with general steps for possible future work in the area.

1.1 Linear Models

Assume a regression model with q independent variables, each of which has n observations. The general form of the regression model is given by

$$y_t = f(\mathbf{x}_t, \boldsymbol{\beta}) + \varepsilon_t, \quad i = 1,2,...,q, \quad t = 1,2,...,n \tag{1.1}$$

In (1.1), the response y_t depends on two parts, the deterministic part and the stochastic part. The deterministic part is represented by the function $f(\mathbf{x}_t, \boldsymbol{\beta})$, which is a function of the vector of predictor variables $\mathbf{x}_t = (x_{1t}, x_{2t}, ..., x_{qt})$ and the parameters vector $\boldsymbol{\beta}$. The stochastic part is represented by the regression errors ε_t, $t = 1,2,...,n$. The error term is defined as the amount of deviation in y_t from its expected value, where ε_t is usually assumed to be a set of independent and identically normally distributed random variables with mean zero and constant variance σ_ε^2.

The function $f(\mathbf{x}_t, \boldsymbol{\beta})$ in (1.1) could be linear or nonlinear in parameters. If it is linear, the model is termed a linear response model. As an example, assume that we have n observations, denoted by y_t, $t = 1,2,...,n$ and that the errors ε_t are additive. Then, a simple linear model can be given by

$$y_t = \beta_0 + \beta_1 x_t + \varepsilon_t, \tag{1.2}$$

where the parameters β_0 and β_1 in the model are called regression coefficients. The parameter β_1 is the slope of the regression line. It indicates the amount of change in y that happens as a result of a unit change in x. The parameter β_0 is the intercept of the line.

When the response variable depends on several explanatory variables, the model is called a multiple linear regression. For example, consider a model with two predictors x_1 and x_2. The model may have the form, $y_t = \beta_0 + \beta_1 x_{1t} + \beta_2 x_{2t} + \varepsilon_t$.

The objective of many studies is to develop appropriate models in order to estimate and make inferences about the unknown parameters. These parameters are estimated using a sample of n observations. There are different ways to estimate the parameters, such as, the ordinary least squares (OLS) method (Draper & Smith, 1981), which depends on minimizing the error sum of squares. This minimum is found by differentiating the error sum of squares with respect to the model parameters, and equating these derivatives to zero, which gives the estimating equations. The values of the parameters that satisfy these equations are known as the least squares estimates. The variance of the OLS estimator is considered to be the minimum variance among all the estimates, and if the model is correct, the least squares estimators are asymptotically unbiased and efficient estimators. The estimators can then be used to predict the values of the response given selected observations of the predictor variables.

The OLS approaches is useful to be used when the assumption of constant response error variance holds. However, this assumption may not be valid in many practical situations. In this case, an alternative method, weighted least squares (WLS) (Draper & Smith, 1981) can be used. The method is similar in application to the OLS approach but only the response variance at each observation is assumed to be known (or accurately estimated), and used in the analysis. Both OLS and WLS will be used in Chapter 3 as methods of analysis, and both are available in the R software package. However, we only use the built-in function 'lm' in R to carry out the OLS method. For WLS, we develop suitable code for the case under study.

Other approaches can also be used for the estimation, such as maximum likelihood estimation (MLE) (Kendall & Stuart, 1961). The basic idea of MLE is to write down the likelihood function, which is the probability of obtaining the sample data given the probability distribution model. This function contains the unknown model parameters. The values of the parameters that maximize the likelihood function are the maximum likelihood estimators.

1.2 Nonlinear Models

Nonlinear models are often used when the parameters of the model have a known interpretation, that is, each parameter in the nonlinear model explains a certain mechanism in the experiment under study. The general form of the nonlinear model can be interpreted as (1.1) where the function $f(\mathbf{x}_t, \boldsymbol{\beta})$ is nonlinear in both $\mathbf{x}_t$ and $\boldsymbol{\beta}$. These models have several advantages over linear models. For example, the number of parameters in nonlinear models is usually less than that in linear models (Atkinson & Donev, 1992). Therefore, nonlinear models are considered to be more suitable than linear models for practical situations. However, estimating their parameters can be a complex procedure.

Consider a response model with p parameters. The value of the error sum of squares is given by

$$\eta = \sum_{t=1}^{n} [y_t - f(\mathbf{x}_t, \boldsymbol{\beta})]^2 .$$

Now to establish the least squares estimates of β_j, $j = 1,2,\ldots,p$, η is differentiated with respect to β_j, for $j = 1,2,\ldots,p$. Then, the estimating equations are obtained by equating $\partial\eta/\partial\beta_j$ to zero, and $\hat{\beta}_j$ are the least squares estimates that satisfy these equations

$$\frac{\partial \eta}{\partial \beta_j} = \sum_{t=1}^{n} [y_t - f(\mathbf{x}_t, \boldsymbol{\beta})] \frac{\partial f(\mathbf{x}_t, \boldsymbol{\beta})}{\partial \beta_j}\bigg|_{\beta=\hat{\beta}} = 0 , \qquad j = 1,2,\ldots,p . \tag{1.4}$$

Let $\nabla(\mathbf{x}, \boldsymbol{\beta})$ denote the $n \times p$ matrix whose (t, j) element is $\partial f(\mathbf{x}_t, \boldsymbol{\beta})/\partial\beta_j$, thus the estimation equations (1.4) become

$$\nabla^T(\mathbf{x}, \boldsymbol{\beta})\mathbf{y} = \nabla^T(\mathbf{x}, \boldsymbol{\beta})\mathbf{f}(\mathbf{x}, \boldsymbol{\beta}) , \tag{1.5}$$

where equation (1.5) is the same as the normal equations obtained in the OLS method. However, both ∇ and $\mathbf{f}(\mathbf{x}, \boldsymbol{\beta})$ depend on the unknown model parameters $\boldsymbol{\beta}$. Hence, unlike in the linear least squares method, there is no analytical solution for the estimators. To find

approximate solutions, iteration techniques are used to estimate the parameters and to obtain the nonlinear least squares (NLS) estimates. An iteration requires the selection of appropriate starting values, denoted by $\boldsymbol{\beta}^0$. The main problem that can occur is $\boldsymbol{\beta}^0$ converging to a false value or not converging at all (non-convergence). Such problems can happen for a variety of reasons. For example, $\boldsymbol{\beta}^0$ may not have been chosen well in the beginning, and thus could be too far away from the true value, the data may contain outliers, or the model may not be appropriate for the data, to mention just a few possible reasons (Ratkowsky, 1990). The initial value $\boldsymbol{\beta}^0$ can be chosen either by plotting the relationship between the response and the regressor or by reasonable guess.

Different iteration techniques include the Gauss-Newton, Steepest descent, and the Simplex method. All depend on iteratively improving $\boldsymbol{\beta}^0$ using specific optimization criteria. For example, the Gauss-Newton algorithm depends on iteratively minimizing the residual sum of squares at each iteration step, until it reaches a small enough value. The idea behind this is to assume that the function $f(\mathbf{x}_t, \boldsymbol{\beta})$ is approximately linear near the least squares estimators.

The value of $\boldsymbol{\beta}^0$ is assumed to be a good approximation to $\hat{\boldsymbol{\beta}}$ so it can be used to start off the iterative procedure. The linearization can be done using first order Taylor series expansions or the delta method (Meyer, 1965, p.128). The number of iterations is not important but some of the methods require a large number of iterations in order for their estimators to achieve certain asymptotic properties. For a full explanation, see Bates and Watts (1988), Seber and Wild (1989), and Huet et al. (2004).

In Chapter 4, we will use a direct application of the Gauss-Newton method to estimate the parameters of the chosen nonlinear model. This is available as a built in function ‘nls’ in the R software package. Furthermore, the weighted nonlinear least squares (WNLS) method (Bates & Watts, 1988) is used for the analysis of data.

2 The Problem of EIV and the Literature Review

2.1 EIV Definition

Errors-in-variables (EIV) are deviations of observed measurements from their true (or target) values. In regression models, if the fitted data contains a predictor variable measured with error, it is customary to describe the models as measurement error or EIV models. These models contain two predictor variables. The observable predictor is observed in the study and is associated with an error, while the true or actual variable is known as the unobservable or error-prone predictor. Usually the value of the unobservable variable is unknown, otherwise it would be used to produce an actual (or true) analysis of data. Hence the observable predictor is used to fit the regression model.

In practice, EIV are likely to occur for many reasons. In studies requiring data collection over a long period of time, accurate measurements can require complex and expensive procedures. Errors can occur due to a fault in the device used to read observations, a biological condition, and/or an error made by the experimenter while collecting the data; see, for example Rabinovich (2006, p. 20).

2.2 Types of EIV

There are many types or scenarios of EIV. Important distinctions between scenarios involve how the errors occur and what distributions they follow. For example, if an explanatory variable x cannot be measured directly or precisely, the values used in the statistical analysis denoted by w are

$$w_t = x_t + u_t, \qquad t = 1,2,\ldots,n, \tag{2.1}$$

where n is the number of observations. Different assumptions can be made about the so-called *classical* additive error u, for example they are often assumed to be independently and identically normally distributed random variables with mean zero and constant variance σ_u^2, i.e. $u \sim N(0,\sigma_u^2)$, independent of the unobservable regressor x and correlated with the observed regressor w,

$$\operatorname{cov}(w,u) = E[wu] - E[w]E[u] = E([x+u]u) = E[xu] + E[u^2] = \sigma_u^2.$$

Typically, classical measurement errors are encountered in observational studies. For example, in a study of cardiovascular disease, measurements of the cholesterol level in the blood (as a risk factor) often include errors.

However, when the data are collected in a designed experiment and specified by the experimenter, the fixed design values w are set with errors, i.e.

$$x_t = w_t + u_t, \qquad t = 1,2,\ldots,n. \tag{2.2}$$

The inaccurate values w_t are usually used in the estimation of the statistical model of the data, because the values x_t are unknown. The errors in this case are known as *Berkson* errors, named after Berkson (1950) who was the first to study this error structure. Unlike the assumptions of model (2.1), here the error u is independent of the predictor w and correlated with x, i.e. $\operatorname{cov}(x,u) = \sigma_u^2$. Thus the values x_t used to generate the responses in the experiment are no

longer fixed design points. If $u \sim N(0, \sigma_u^2)$, it is often assumed that, on average, the values of the true predictor converge to the design values, i.e.

$$E(x_t \mid w_t) = w_t, \qquad t = 1,2,\ldots,n, \tag{2.3}$$

Such an assumption has to be verified, usually with a validation experiment. In the majority of cases, it is expected that the measurement errors have zero mean. However, they could have a non-zero mean (Donev, 2000, p. 2068). The variance of the EIV can be known or unknown. In the case where it is unknown, it can be estimated in different ways, depending on the case under study. For example, the estimation of the error variance in classical models is usually achieved by taking independent replications of the variable measured with errors. Note that in (2.1) and (2.2), u_t, $t = 1,2,\ldots,n$, are called homoscedastic errors if σ_u^2 is constant for $t = 1,2,\ldots,n$, and heteroscedastic errors otherwise. For example, in model (2.2), the error variance σ_u^2 could be proportional to a function of the design points, i.e. $\varphi(w_t)\sigma_u^2$, $t = 1,2,\ldots,n$, where $\varphi(w_t)$ defines the way the variance changes with *w*. Hence, the variance in the actual values x_t is $\varphi(w_t)\sigma_u^2$, $t = 1,2,\ldots,n$.

In the presence of EIV, the results obtained from regression analysis may not be valid if the errors are ignored. Statistical analysis of the data that ignores these errors is called naive analysis. To obtain a corrected analysis, the effects of EIV should be taken into account. These effects depend on many factors, as will be seen later. For instance, the more complicated the model, the more difficult it is to explain and understand the effects.

The problem of EIV has received considerable attention in the literature. Monographs summarizing the methods available for handling such data include Fuller (1987), Cheng and Van Ness (1999) and Carroll et al. (2006). The main target of these studies is to analyze and reduce (or eliminate in some cases) the effects of ignoring the EIV on the parameter estimates and the response prediction. The effects include biased (or inconsistent) and/or inefficient

estimators of regression parameters, which results in poor inferences about confidence intervals and the hypothesis testing of parameters. Here, we provide a short review of some of the most common results, using the simple linear regression model in equation (1.2). We use model (1.2) for its simplicity, which allows us to interpret the results and provide a clear understanding of the consequences and effects of EIV. The review is based on Fuller (1987) and Carroll et al. (2006).

Assume classical EIV represented by equation (2.1). We aim to estimate the slope β_1 in model (1.2). The OLS approach is customarily used to obtain the minimum variance unbiased estimator. If the predictor variable were measured without error, the actual OLS estimator $\hat{\beta}_{1,actual}$ would be an asymptotically unbiased estimator for β_1 (Draper & Smith, 1981). However, when *w* is observed instead of *x*, with $u \sim N(0, \sigma_u^2)$, and the regression error ε is independent of *u*, the naive OLS estimator $\hat{\beta}_{1,naive}$ is the asymptotically unbiased estimator for $\sigma_x^2(\sigma_x^2 + \sigma_u^2)^{-1}\beta_{1,true}$, where the ratio $\sigma_x^2(\sigma_x^2 + \sigma_u^2)^{-1}$ is called the reliability ratio, and σ_x^2 is the variance of the actual variable *x* (Fuller, 1987). The reliability ratio is also called the attenuation factor since it causes the value of the naive estimator to approach zero, and hence β_1 is underestimated. Consequently, the relationship between the response variable and the regressor will be also underestimated.

Addressing the form of the bias in naive estimators is always important to give a clear representation of the effect of the EIV. For example, the attenuation in $\hat{\beta}_{1,naive}$ is small, with a variation in the true predictor that is larger than the value of the error variance.

The slope estimator is not the only estimate that will be affected by a measurement error in the predictor, the OLS estimator of the intercept will also be biased (Fuller, 1987). Often, the bias is a function of the error variance and the model parameters. Therefore, as the measurement error variance increases, the bias in both estimates will increase as well. Since

the regression model is used to predict the response y, this value will be biased if it is based on biased estimates (Hodges & Moore, 1972).

In addition, EIV increase the response variance. For example, by rewriting model (1.2) and considering the classical error model (2.1), the response can be given by

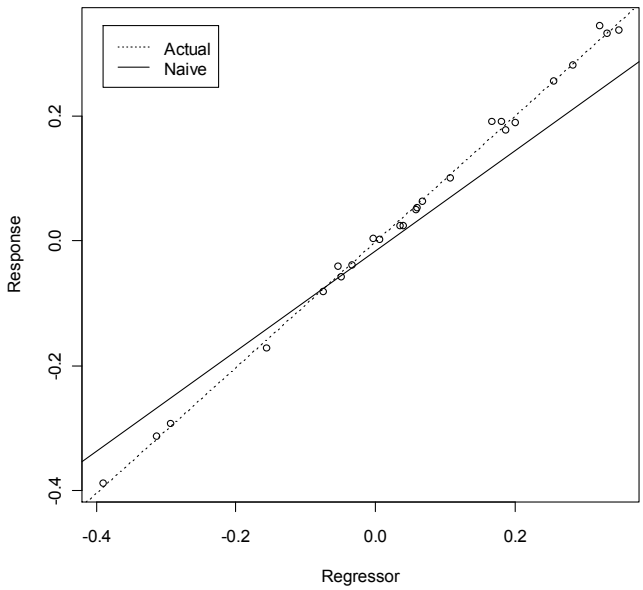
$$\begin{aligned} y &= \beta_0 + \beta_1(w-u) + \varepsilon \\ &= \beta_0 + \beta_1 w + (\varepsilon - \beta_1 u), \end{aligned}$$

and thus the error term now has variance

$$\text{var}(\varepsilon - \beta_1 u) = \text{var}(\varepsilon) + \beta_1^2 \text{var}(u) = \sigma_\varepsilon^2 + \beta_1^2 \sigma_u^2 > \sigma_\varepsilon^2,$$

which results in the data being more widely scattered around the regression line. Figure 2.1 summarizes all the reviewed results regarding the effect of classical EIV on regression analysis using model (1.2), showing the differences between the naive and actual fits of the data.

Figure 2.1 The effects of classical errors on regression analysis using simple linear response model, $x \sim N(0, 0.063)$, $u \sim N(0, 0.01)$, and $\varepsilon \sim N(0, 1\times10^{-4})$.



Not all of the effects we have discussed above hold for all types of response models. For example, when Berkson errors in eq. (2.2) occur in the variable of model (1.2), model (2.3) holds and as a result $E[y \mid w] = \beta_0 + \beta_1 w$. Therefore, the EIV in such a case do not lead to

biased estimates of the parameters of model (1.2) but they do inflate the response variance (Fuller, 1987). With Berkson EIV, unbiased estimates of the coefficients are a typical result found in linear and nonlinear models. For example, in linear response models, Box (1963) studied the effect of Berkson errors on the naive analysis of polynomial models. He used factorial designs to illustrate those effects. Box concludes that Berkson errors lead to biased models and unbiased estimates of the coefficients, with an increase in the response variance. The unbiasedness in the naive estimates is due to the fact that the bias in $E[y \mid w]$ is constant between the experimental runs. In nonlinear response models, studies by Rudemo et al. (1989) and Racine-Poon et al. (1991) conclude that there is no bias or negligible bias in the estimates of the coefficients, but that their variances were inefficiently estimated. However, when linear or generalized linear models have to be estimated, studies by Burr (1988), Whittemore and Keller (1988), Buonaccorsi and Lin (2002) and Kim et al. (2006) showed that Berkson errors may lead to bias in the estimates of the model parameters. In these cases, the bias was mostly seen to be important with large EIV.

Complex Berkson error structures can also lead to biased estimates of the parameters. For example, Küchenhoff et al. (2007) compared the effects of additive (as shown in model (2.2)) and multiplicative ($x = w \times u$) Berkson errors on the estimation of the slope of a Cox proportional hazard model. They found that an additive Berkson error can cause a slight attenuation in the estimate of the slope parameter. This is not true in the case of multiplicative Berkson errors as the estimators adapt to the larger bias. However both errors are found to influence the efficiency of the estimators by underestimating their true variances. The bias is found to be a function of the model parameters–a monotonic increasing relation is observed by Küchenhoff et al. between the magnitude of the slope and the attenuation in its estimator.

Another possible effect of EIV, whether classical or Berkson, is heterogeneity (or heteroscedasticity) in the response variance. This means that the response variance depends on

the values of the predictor, and its value is no longer a fixed constant σ_ε^2. Heterogeneity can be affected by different factors, such as the type of model (linear or nonlinear), and/or the error structure (homogeneous or heterogeneous). It is a major violation of the general rules of any regression analysis and ignoring it in the analysis can affect the efficiency of the estimators of the model parameters (Draper & Smith, 1981). For example, the standard errors of the estimators could underestimate or overestimate the estimators' true variability.

The number of variables in the response model also plays a major role in the effect of EIV. When the regression model has more than one predictor, and multiple predictors are measured with errors, interpreting the effects is complicated and even unpredictable in some cases. For example, it is possible that if at least one variable is measured with error, then all the estimates of the coefficients will be biased, and in this case the direction of the bias could be unpredictable (Buzas et al., 2004). The biases may increase if the correlations between the predictors and the measurement error variances are high (Carroll et al., 1985; Sturmer et al., 2002; Carroll et al., 2006; Yuan, 2007).

Moreover, EIV leads to a loss of power in hypothesis testing using the regression model parameters and this applies to both classical and Berkson errors. In addition, the tests loses more power when the EIV are larger. The sample size has to be enlarged in order to gain the same level of power that would be gained from an EIV-free model. This is illustrated in Carroll et al. (2006, pp. 18-19), using a simulation study carried out by plotting the amount of EIV variance against the power of the hypothesis testing using the regression slope.

The effects of EIV can be corrected or adjusted using various approaches. In the following section, we review some of the established methods used to correct the bias in naive estimates, under the assumption of classical EIV. In the literature on EIV, the most well-known correction methods have been developed to deal with classical errors. These methods

are sometimes described as bias-based correction approaches as they eliminate or reduce the bias in the naive estimates.

2.3 Best-Known Correction Methods for Classical EIV

The correction methods for EIV can be classified into structural and functional approaches. Structural approaches assume that the true predictor is a random variable that follows a specific distribution. If x is assumed to be a fixed design point then a functional method is used. Functional models are also used when x is a random variable, but no assumptions are made about its distribution. This classification was first introduced by Kendall (1951, 1952) and is an important part of choosing the appropriate method for estimating the model parameters.

Structural estimation methods require the specification of the distribution function of x. Information on x can be gathered either from preliminary samples or data from previous studies.

In practice when correcting for the effect EIV, information about the distribution of x is not always available, hence the assumption in functional methods that x is fixed simplifies the problem as no distributional assumptions need to be made. However, the functional methods are considered less robust in dealing with the effect of EIV than structural methods since they depend mostly on approximate solutions. For example, the maximum likelihood approach is a well-known robust way of estimating the parameters but it requires the distribution of x to be known. The method has been used only occasionally in the literature on EIV because of its computational complexity and the large number of assumptions that need to be satisfied (Guolo, 2008; Guolo & Brazzale, 2008).

Structural models have received attention as well. Thompson and Carter (2007) provided a comprehensive review of EIV models, focusing on structural models with a normality

assumption about the distribution of the true regressor. Fuller (1987) studied structural models and pointed out that different estimating approaches can be used depending on the distribution of the unobservable variable x.

In addition, the distribution of the EIV plays a significant rule in specifying the method of correction. Practitioners and statisticians have focused on the cases where the EIV are independently and normally distributed. The assumption of the normality of the errors is useful if it can be made, because it is important for the robustness of most of the correction methods.

Researchers have been trying to eliminate the need for any distributional assumptions for the EIV or the true variables. For instances, Liu and Liang (1992) proposed a method for obtaining consistent estimates which depends on replicate measurements. They also provide an approach to be used for choosing an adequate number of replicates. They point out that their suggestion could be difficult to apply in practice, however, as making replicate measurements could be difficult and incur high costs. Spiegelman (1994) has reviewed some of the better study designs that can be used with nonlinear EIV models and also considers the costs of such designs.

Some other methods require the use of validation data. For example, Lee and Sepanski (1995) proposed a method for improving the estimates of linear and nonlinear EIV models, based on the availability of validation data prior to the study. Carroll et al. (2010) proposed an approach that requires neither validation data nor any information about the actual independent variable in the model.

A method called the instrumental variable (IV) is also useful when no information on the EIV is available. In this approach, an additional variable (the IV) is introduced into the model. For example, the IV could be the value of a second observation of the true variable x, derived in a different way from the first one. The IV has to satisfy various assumptions, for example, it

must be correlated with x and independent of the measurement error. Details of estimations using IVs in linear and nonlinear models can be found in Fuller (1987) and Carroll et al. (2006, chap. 6).

Another correction method that has been discussed in the literature is the method of moments. This method is considered to be the simplest correction technique and it can be used if the measurement error variance σ_u^2 is known or can be accurately predicted, and the form of the bias in the naive estimates can be identified (Fuller, 1987). It aims to eliminate the effect of EIV on the results and parameter estimation by producing an estimate using the moment estimator, which performs better than the naive estimator. This can be seen by recalling a simple linear regression with an estimated reliability ratio. Assume the variance of the observed values of the predictor measured with errors is known. The corrected slope estimate $\hat{\beta}_c$ can be calculated using the formula

$$\hat{\beta}_c = \frac{\sigma_w^2}{\sigma_x^2}\hat{\beta}_{naive} = \frac{\sigma_w^2}{\sigma_w^2 - \sigma_u^2}\hat{\beta}_{naive}.$$

In the following, we comprehensively review two of the best-known approaches for correcting the bias in the estimates of regression coefficients when classical errors occur and, in some special cases, when both classical and Berkson errors occur. These approaches are regression calibration and simulation-extrapolation.

2.3.1 Regression Calibration

Regression calibration (RC) is a simple way of adjusting for the effect of EIV. It has been extensively used in the literature on EIV models, for example, in epidemiology studies with Cox proportional hazards models (Prentice, 1982), in generalized linear models (Armstrong, 1985; Rosner et al., 1989; Schafer, 1990; Kuha, 1994), and in nonlinear response models (Gleser, 1990). The basic idea of RC is to replace the observed predictor in the regression model with an unbiased estimate of the true value. The estimate can be found in

several ways, but the most common is to approximate the conditional expectation of the true predictor given the observed value $E[x \mid w, z]$, where z is any other covariate in the model (Carroll et al., 2006). For example, if the response model is a simple linear regression (1.2), the RC estimator is found by estimating the coefficient in the regression $y = \beta_0 + \beta_1 E[x \mid w] + \varepsilon$.

Estimating the expectation $E[x \mid w, z]$ is described as an "art" in Carroll et al. (2006) as it requires a full understanding of the EIV model. The RC approach is considered to be either a functional or a structural method of correction, depending on the assumptions needed to estimate $E[x \mid w, z]$. For example, the method is structural if the estimation of $E[x \mid w, z]$ requires additional information about the distribution of the unobserved variable x. No specific assumptions regarding the EIV have to be satisfied. Appropriate assumptions depend on the case under study. For example, Rosner et al. (1989) assumed a small EIV in order to obtain a good approximation for $E[x \mid w, z]$, while Kuha (1994) in an extension of the work of Rosner et al. (1989), has relaxed this assumption, and also assumes that only some of the moments of the distribution of the errors are known.

The form of $E[x \mid w, z]$ is not unique; it depends on the type of information available in the study. The OLS estimates from fitting the response y on $E[x \mid w, z]$ are approximately unbiased since this expectation is only an estimate of the actual unobserved variable. To find the expectation of the actual variable given the observed value, x can be written as a function of w and then Taylor series expansions used to obtain $E[x \mid w, z]$ (Carroll & Stefanski, 1990). Validation data or replications could also be necessary. The estimate of $E[x \mid w, z]$ should be as accurate as possible, otherwise using it to fit the response model may lead to biased estimators (Carroll et al., 2006).

Although RC is widely applicable, the consistency of the approach is questionable when dealing with complex nonlinear models, and in most cases a small variance of EIV is required

for the approximation to be satisfactory (Carroll et al., 2006). In such cases, the estimators obtained are only approximations of the true parameters. In addition, heteroscedastic EIV could affect the robustness of the approach. For example, if the variance of the true value, given the observed one, $\text{var}(x \mid w)$, is heteroscedastic, RC may only be useful for correcting the bias in naive estimates; however, it does not provide efficient standard errors of the parameter estimates (Carroll et al., 2006). This may occur when dealing with Berkson errors since they are usually heteroscedastic by nature, that is, the error in the input of a design is mostly correlated to the design value.

As with most correction methods, using the RC approach to correct the bias in naive estimates may introduce more uncertainty into estimates of model parameters. The uncertainty comes from using an approximate estimate of $E[x \mid w, z]$ instead of the actual value, and this can be compensated for by using the bootstrap technique to obtain the standard errors of the RC estimators (Carroll & Stefanski, 1990). The bootstrap method is a nonparametric approach for making inferences about the parameters of the sample under study, when the parameters have unknown probability distributions (Efron, 1982). It can be used to estimate the biases and standard errors in the parameter estimates. The methodology is based on randomly generating new samples from an original available sample. Each sample is of the same size as the original one and is called a bootstrap sample. The target statistical characteristics are computed for each sample. The number of samples should be sufficient to approximately attain the target parameters of the study, and to reduce the variability in the observations (Efron & Tibshirani, 1993).

2.3.2 Simulation-Extrapolation

The simulation-extrapolation (SIMEX) approach was proposed by Cook and Stefanski (1994) as a functional correction tool for EIV effects on linear and nonlinear models in

epidemiological studies. It produces the so-called SIMEX estimator which is then used to correct the bias in the naive parameter estimates. The algorithm simulates two types of model, an additional measurement errors model and an error-free model. The SIMEX estimator is the estimate found as a result of fitting the error-free model, which is an approximation of the actual model.

The algorithm is based on estimating the regression coefficients through a process of adding errors with increasing variances to the observable variables that have been measured with errors. The relationships between the error variances and the estimated coefficients are fitted for each coefficient in the model and then extrapolated to the ideal case where there is no EIV.

In order to illustrate how SIMEX works, we consider the following example. Assume we have a response model with parameter vector given by $\boldsymbol{\beta}$. Assume the classical error model (2.1), where u_t are independent identically normally distributed errors with mean zero and known constant variance σ_u^2, i.e. $u_t \sim N(0,\sigma_u^2)$, and x_t is the actual *t*th measurement on a continues variable *x*, follows known or unknown distribution. For $\lambda \geq 0$, *n* new observations of the observed predictor are generated *B* times,

$$w_{b,t} = w_t + \lambda^{1/2}\sigma_u u_{b,t}, \quad t = 1,2,\ldots,n, \quad b = 1,2,\ldots,B,$$

where λ is called the multiplication factor, and reflects the amount of additional error added to the observed variable, $u_{b,t}$ are independent identically distributed normal random variables with mean zero and variance one, i.e. $u_{b,t} \sim N(0,1)$, σ_u is the standard deviation of the measurement error, and B is the number of samples being generated in the simulation process. In practice, most of the time the values of λ are in the range [0, 2]. Any number *B* can be chosen, but it should be large enough to guarantee the consistency of the SIMEX estimator (Stefanski & Cook, 1995). The method is usually applied with the assumption of

standard normally distributed measurement errors but this assumption is not essential (Carroll et al., 1996). The values $\lambda^{1/2}\sigma_u u_{b,t}$ are generated and added to the observed predictor *B* times for each value of λ. Note that the total measurement error variance for the *t*th set of observations is

$$\text{var}(w_{b,t}(\lambda) \mid x_t) = \sigma_u^2 + \lambda\sigma_u^2 = (1+\lambda)\sigma_u^2 . \tag{2.4}$$

So when λ=0, the model is free from additional errors but still contains the original EIV (naive model) and the estimate found at that point is the naive estimator $\hat{\boldsymbol{\beta}}_{naive}$.

The new values of the predictor, that is, the values with additional errors, are analyzed by finding the regression parameter estimates $\hat{\boldsymbol{\beta}}_b(\lambda)$ of regressing y on $w_{b,t}$ for each value of λ, where $\hat{\boldsymbol{\beta}}_b(\lambda)$ is a vector of the estimates of the model parameters, for $b = 1,2,\ldots,B$. Any estimation approach can be used, for example, the OLS method. After obtaining the estimates $\hat{\boldsymbol{\beta}}_b(\lambda)$, the average of these estimates is computed for each λ using

$$\hat{\boldsymbol{\beta}}(\lambda) = \frac{1}{B}\sum_{b=1}^{B}\hat{\boldsymbol{\beta}}_b(\lambda), \qquad \lambda \geq 0 .$$

The main purpose of averaging over the obtained estimates is to reduce the amount of variation associated with the simulation process. As a result, different estimates are obtained for different values of λ.

Since $\hat{\boldsymbol{\beta}}(\lambda)$ is a function of λ, a functional relationship can be specified, and used to perform an extrapolation at a certain value of λ. Cook and Stefanski (1994) used three types of fitting functions: the simple linear model given by

$$\hat{\boldsymbol{\beta}}(\lambda) \approx a_0 + a_1\lambda , \tag{2.5}$$

the quadratic model

$$\hat{\boldsymbol{\beta}}(\lambda) \approx a_0 + a_1\lambda + a_2\lambda^2 , \tag{2.6}$$

and the nonlinear model

$$\hat{\boldsymbol{\beta}}(\lambda) \approx a_0 + \frac{a_1}{a_2 + \lambda}. \tag{2.7}$$

The unknown parameters a_0, a_1, and a_2 in models (2.5) and (2.6) can be estimated by the least squares approach. For the nonlinear extrapolate (2.7), the nonlinear least squares approach can be used, which requires specifying initial values for a_0, a_1, and a_2. One way of finding the initial values is to first fit model (2.6). Then the initial values can be obtained from a three-point fit to 0, $\lambda_{\max}/2$ and $\lambda_{\max}$ and the predicted values from the fitted model (2.6) at these three points (Carroll et al., 2006, p.110).

The extrapolation in SIMEX is established by setting λ = -1 in the fitted model for each component of the vector $\hat{\boldsymbol{\beta}}(\lambda)$ so that, from equation (2.4), the total measurement error variance in $w_{b,t}$ is zero. This means that an estimate is obtained for the case when the data is free from any type of error (additional or original measurement error). It may seem as though errors with a negative variance are being added. However, the negative sign in SIMEX can be explained as a "hypothetical case", only used to perform the extrapolation (Carroll et al., 2006, p. 102).

Note that, the extrapolation process depends on the function that has been assumed to fit the data and, as mentioned earlier, the exact function is usually unknown. Therefore, the SIMEX estimator is an approximately consistent estimator of the estimator obtained from the actual analysis, that is

$$E(\hat{\boldsymbol{\beta}}_{simex}) \approx \hat{\boldsymbol{\beta}}_{actual}. \tag{2.8}$$

We have described the SIMEX approach for the case of homogeneous measurement errors. However, the same methodology can be applied when the errors are heteroscedastic, by plugging the individual variances of the errors $\sigma^2_{u,t}$ into the *t*th observation instead of σ^2_u (Carroll et al., 2006, pp. 102-103).

Researchers have proposed various modifications to the SIMEX approach to be used in situations where the original SIMEX does not work or is not the most appropriate solution. Devanarayan and Stefanski (2002) proposed a technique called empirical SIMEX, modifying the original SIMEX. The original SIMEX approach can be applied if the measurement error variance is known or can be predicted using available observations. On the other hand, the empirical SIMEX can be applied if the measurement errors are heteroscedastic with unknown variances, but replications are available.

As described above, SIMEX can be used to correct for EIV in continuous variables. However, if EIV affects a categorical variable, such as gender, then the variable is said to be misclassified. To correct measurement errors in misclassified variables, the usual SIMEX approach cannot be used, and a modified SIMEX approach called misclassification simulation-extrapolation (MC-SIMEX) must be applied instead (Küchenhoff et al., 2006).

SIMEX is known for its practicality. It does not require a strong mathematical framework, and needs fewer distributional assumptions than most of the other correction methods. However, it does involve a large amount of computational effort, which can be time consuming in a lot of cases.

SIMEX is a built-in function in the R software package and in other packages, such as STATA. In the following example, we illustrate how SIMEX works to correct the bias in the naive estimates when homogeneous errors occur in the predictor variable of the simple linear model given in equation (1.2). Note that we do not use the built-in function in R but instead develop SIMEX code which can be used for different purposes, as will be seen later.

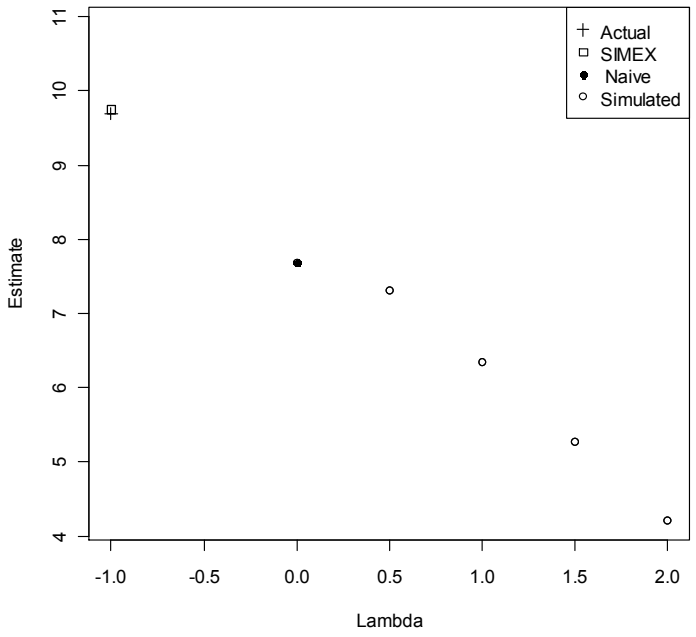
2.3.3 Simulation Example

Assume B=10, n=1000 and observations are generated using $u \sim N(0,0.5)$, $x \sim N(0,1)$ and $\varepsilon \sim N(0,0.2)$. In the simulation step, the multiplicative factor λ is given values of 0, 0.5,

1, 1.5 and 2. The parameters β_0 and β_1 in the simple linear model (1.2) are set to zero, and 11.263, respectively, so we only interested in the effect of the EIV on the slope estimates. Assume model (2.6) is used for the extrapolation step of SIMEX. Then the results show that the actual and naive slope estimates are 11.250 and 9.130, respectively. The SIMEX estimate is 11.592. Hence, using SIMEX reduces the bias in the naive estimate of β_1. The SIMEX algorithm can be represented graphically by modelling the relation between $\hat{\boldsymbol{\beta}}(\lambda)$ and λ.

Figure 2.2 provides evidence of the improvement by the SIMEX estimator over the naive one. The actual OLS estimates are also shown, for the purpose of comparison. The horizontal axis shows λ, which controls the increasing amount of error variance, and the vertical axis represents the values of the estimates using the actual, SIMEX and naive approaches. It can be seen that as the error variance increases, larger differences appear between the naive and SIMEX methods, while the latter produces an estimator that approximate the actual estimator.

Figure 2.2 A comparison between the actual OLS, naive OLS, and SIMEX estimates.



Note that in the above illustration we used a model with a single predictor variable. However, SIMEX can be applied simultaneously for any number of predictors. The SIMEX

fitting function has to be selected carefully since it influences the statistical properties of the SIMEX estimator. Plotting the relationship between $\hat{\boldsymbol{\beta}}(\lambda)$ and λ can be useful for choosing an appropriate function. Carroll et al. (2006) described the quadratic extrapolation model as more 'stable' than nonlinear extrapolation, especially in the case of large errors. On the other hand, there are cases in which the differences between models (2.5), (2.6), and (2.7) are negligible (Buzas et al., 2004). The extrapolation is only one step in the entire analysis. The size of the extrapolation depends on the size of the error variance, with a larger error variance resulting in a larger extrapolation step, and thus a potentially larger approximation error. For this reason, SIMEX is more effective when the EIV are not large.

The distributional properties of the SIMEX estimator have been investigated by Carroll et al. (1996) and Küchenhoff et al. (2007). Under the assumption of normally distributed EIV, they find that the SIMEX estimator is asymptotically normally distributed. The mean of the SIMEX estimator is assumed to converge to the actual estimator, which is assumed to be unbiased for the true parameter as $B \to \infty$. The variance of the SIMEX estimator can be obtained through various approaches, but they are complex, despite the fact that SIMEX is easy to use. To estimate the variance, the bootstrap approach, estimating equations, and the simulation-extrapolation information variance estimation approach can all be used.

The bootstrap approach requires a large amount of computational effort, which can be time consuming in many cases. If the bootstrap method is used to find the variance of the SIMEX estimator, the computational burden can increase still further, since SIMEX itself generally requires the simulation of a large number of samples. The estimating equation approach was proposed by Carroll et al. (1996), and is explained in detail in Carroll et al. (2006, pp.395-398). Since the approach is quite complicated and requires a great deal of computational effort, we do not describe it here.

The information variance estimation approach was proposed by Stefanski and Cook (1995). The approach can be used with the assumption of known error variance, as well as heteroscedastic and homoscedastic EIV. It has been noted that the method performs well with small error variance (Greene & Cai, 2004) and with a large sample size (Battauz et al., 2008). The method is discussed in detail in Carroll et al. (2006, pp. 393-395). Here, a brief summary is given. From (2.8), it follows that

$$Q_{\hat{\boldsymbol{\beta}}_{simex}} \approx Q_{\hat{\boldsymbol{\beta}}_{actual}} + Q_{\hat{\boldsymbol{\beta}}_{simex}-\hat{\boldsymbol{\beta}}_{actual}}, \tag{2.9}$$

where $Q_{\hat{\boldsymbol{\beta}}_{simex}}$ is a $p \times p$ variance-covariance matrix of SIMEX estimators (assuming the number of parameters to be corrected is p), $Q_{\hat{\boldsymbol{\beta}}_{actual}}$ is a $p \times p$ variance-covariance matrix of the actual estimators, and $Q_{(\hat{\boldsymbol{\beta}}_{simex}-\hat{\boldsymbol{\beta}}_{actual})}$ is the variance-covariance matrix of the differences between the SIMEX estimators and the actual estimators.

The estimators in $Q_{\hat{\boldsymbol{\beta}}_{actual}}$ can be obtained by applying the same principles as are used in the SIMEX approach. First, using the inverse information matrix approach, the asymptotic variance-covariance $\hat{Q}_b(\lambda)$ of the estimators, are obtained for each λ and $b = 1,2,\ldots,B$, in the simulation step of SIMEX. Then, for each λ, $\hat{Q}_b(\lambda)$ is averaged over all samples to obtain $\hat{Q}(\lambda)$. By plotting $\hat{Q}(\lambda)$ against λ, the relationship can be extrapolated to the point where $\lambda = -1$, by fitting the appropriate extrapolation function. This gives $\hat{Q}_{\hat{\boldsymbol{\beta}}_{actual}}$ as:

$$\hat{Q}_{\hat{\boldsymbol{\beta}}_{actual}} = \lim_{\lambda \to -1} \hat{Q}(\lambda). \tag{2.10}$$

Similarly, an estimate of $Q_{(\hat{\boldsymbol{\beta}}_{simex}-\hat{\boldsymbol{\beta}}_{actual})}$ can be derived from the information from the simulation step:

$$\hat{Q}_{(\hat{\boldsymbol{\beta}}_{simex}-\hat{\boldsymbol{\beta}}_{actual})} = \lim_{\lambda \to -1} \hat{Q}_{[\hat{\boldsymbol{\beta}}_b(\lambda)-\hat{\boldsymbol{\beta}}(\lambda)]}. \tag{2.11}$$

However, since $Q_{[\hat{\boldsymbol{\beta}}_b(\lambda)-\hat{\boldsymbol{\beta}}(\lambda)]}$ is zero if $\lambda = 0$, and takes on positive values for $\lambda > 0$, it is expected that, for $\lambda = -1$, $Q_{[\hat{\boldsymbol{\beta}}_b(\lambda)-\hat{\boldsymbol{\beta}}(\lambda)]}$ will be negative (Stefanski & Cook, 1995, p. 1251). Hence, equation (2.11) can be rewritten as

$$\hat{Q}_{(\hat{\boldsymbol{\beta}}_{simex}-\hat{\boldsymbol{\beta}}_{actual})} = -\lim_{\lambda \to -1} \hat{Q}_{[\hat{\boldsymbol{\beta}}_b(\lambda)-\hat{\boldsymbol{\beta}}(\lambda)]}.$$

An unbiased estimator of $Q_{[\hat{\boldsymbol{\beta}}_b(\lambda)-\hat{\boldsymbol{\beta}}(\lambda)]}$ is the sample variance-covariance matrix $\hat{Q}_s(\lambda)$ of the estimates $\hat{\boldsymbol{\beta}}_b(\lambda)$, for each λ, computed by

$$\hat{Q}_s(\lambda) = \frac{\sum_{b=1}^{B} [\hat{\boldsymbol{\beta}}_b(\lambda) - \hat{\boldsymbol{\beta}}(\lambda)][\hat{\boldsymbol{\beta}}_b(\lambda) - \hat{\boldsymbol{\beta}}(\lambda)]^t}{B-1},$$

If $B \to \infty$ then, for each λ, $E[\hat{\boldsymbol{\beta}}_b(\lambda)] = \hat{\boldsymbol{\beta}}(\lambda)$, so that,

$$E[\hat{Q}_s(\lambda)] = Q_{[\hat{\boldsymbol{\beta}}_b(\lambda)-\hat{\boldsymbol{\beta}}(\lambda)]}.$$

Thus

$$\hat{Q}_{(\hat{\boldsymbol{\beta}}_{simex}-\hat{\boldsymbol{\beta}}_{actual})} = -\lim_{\lambda \to -1} E[\hat{Q}_s(\lambda)]. \qquad (2.12)$$

To find an approximate estimate of $Q_{(\hat{\boldsymbol{\beta}}_{simex}-\hat{\boldsymbol{\beta}}_{actual})}$, one can use the components of $\hat{Q}_s(\lambda)$ to model an extrapolation function, and extrapolate to the case where $\lambda = -1$. Therefore, from (2.9), (2.10) and (2.12),

$$\hat{Q}_{\hat{\boldsymbol{\beta}}_{simex}} \approx \hat{Q}(-1) - \hat{Q}_s(-1), \qquad (2.13)$$

where $\hat{Q}(-1)$ and $\hat{Q}_s(-1)$ are the estimated variance-covariance matrices from fitting the relation between the components of $\hat{Q}(\lambda)$ and $\hat{Q}_s(\lambda)$, for each λ, and extrapolating the fit to $\lambda = -1$. Often, instead of using (2.13) to find $\hat{Q}_{\hat{\boldsymbol{\beta}}_{simex}}$, the total difference between $\hat{Q}(\lambda)$ and $\hat{Q}_s(\lambda)$, for each λ, is computed and extrapolated back to the case where $\lambda = -1$. Either way, the same results are obtained.

This simulation-extrapolation variance estimation approach is mostly used for its simple and direct application. However, there is no guarantee that the variance estimates are positive (Carroll et al., 2006, p.395). One way to deal with this problem is to change the model used for the extrapolation function (see for example Choi et al., 2006).

The variability of the SIMEX estimator is larger than that of the naive estimator because SIMEX is an approximate approach. This holds for any correction approach that depends on approximate procedures, including RC (Wang et al., 1998; Carroll et al., 2006). This could be a problem during the analysis of data if the major concern is to have estimators with small standard errors, such as when obtaining confidence intervals for parameters with a small sample size (Sturmer et al., 2002).

2.3.4 Comparison between SIMEX and RC

There are different situations wherein the robustness of the RC and SIMEX methods will need to be compared in order to evaluate the best approach to use to adjust for the effects of EIV. Comparison studies on the EIV problem have been carried out by a number of researchers. See, for example, Fung and Krewski (1999), Holcomb (1999), Monleon (2005) and Guolo and Brazzale (2008). Generally these studies have concluded that each approach can be useful, depending on the study in question and the type of information available about the EIV model.

For example, Fung and Krewski (1999) investigated the performance of SIMEX and the RC method when a Poisson regression with multiple predictors is fitted to the data. Most of their results look at the effect of the correlation structure between the predictor variables in the model on the adjusted analysis. The slope estimates are calculated over a number of simulation trials and the average of these estimates are used in the comparisons. Generally, their results show that, under the model assumptions, the RC performs better than SIMEX, as long as the correlation between the predictors is small or moderate. Guolo and Brazzale (2008)

pointed out that it is preferable to use SIMEX over the RC approach when the errors are heteroscedastic, however.

Less mathematical background is needed to apply SIMEX and hence the approach is very easy for general practitioners to implement. However, this comes at a cost since there is no plausible closed-form solution for the SIMEX estimator, except in very simple cases. The RC method, on the other hand, can generally be used to produce a mathematical form for the bias in the naive estimators, which can be a useful means of presentation in some cases, as will be seen later on.

2.4 Further Review and Discussion

Both the classical and Berkson EIV can create serious complications in the analysis of regression models. The classical error model has received the majority of the attention from researchers in the past. Most of the well-known correction techniques have been developed for classical error models. Küchenhoff et al. (2007) pointed out that this is due to the wide range of observational studies in which classical errors occur. In addition, Berkson errors may have less effect on the statistical analysis of most response models than classical errors do. Nevertheless, ignoring Berkson errors could still have serious effects, particularly when the errors have a complicated structure, or when they occur in nonlinear response models.

When Berkson errors occur, the OLS approach to estimating model parameters is very often inadequate. For example, Berkson EIV are mostly proportional to the target values (Huwang & Huang, 2000). Therefore, the response variance could become heteroscedastic due to the structure of the errors. The correction approaches for Berkson errors are mostly based on applying the maximum likelihood approach and Bayesian estimation. There are some disadvantages in using such approaches. They require some knowledge about the exact form of the distribution functions of u and ε, or the moments of their distributions, and they are

usually computationally-intensive (Carroll et al., 2006, p. 205). Other approaches have also been used; see for example Fedorov (1974), Huwang and Huang (2000) and Koul and Song (2009). Most of these are based on identifying the first two moments of the response, given the intended design variable, and then incorporating this information into a least squares or weighted least squares analysis. Such approaches are similar to applying the RC approach when there are classical errors, and they were probably inspired by that approach. However, using these approaches is not always a straightforward task, especially when using a complicated class of nonlinear models and/or when complex EIV assumptions are imposed (e.g. correlated errors).

Not a lot of work has been carried out previously on complex error structures. For instance, errors with a heteroscedastic and dependent nature have been much less investigated in the literature of EIV, as will be seen later on. Such errors can occur in both observational and designed studies. The lack of investigation may be because their effects on the analysis of regression models can be difficult to interpret and understand. Moreover, in most real life situations, practitioners are unaware of the existence of these errors when collecting the data. Recently, more studies have been focusing on these EIV assumptions. Studies such as Thamerus (1997), Cheng and Riu (2006), Zavala et al. (2007), Guolo and Brazzale (2008), Wang et al. (2010), and Carroll et al. (2010) all examined the assumption of heteroscedastic classical errors. Xiao et al. (2010) studied correlated classical errors.

Heteroscedastic Berkson errors have received a noticeable portion of attention, especially in bioassay and dose-response studies, such as Rudemo et al. (1989), Racine-Poon et al. (1991), Higgins et al. (1998), and Dellaportas and Stephens (1995). Reeves et al. (1998) considered both Berkson and classical heteroscedastic errors. Some of these studies and others, such as Steiner and Hamada (1997), and Hamada et al. (2005) also considered the situation where the EIV are correlated.

Based on our literature review of the studies on heteroscedastic dependent Berkson errors, we believe that more investigation is needed to examine all the aspects of the effects of these errors. The following chapters introduce a review that we have undertaken to address the important gaps in the related studies mentioned above. We focus on cases wherein heteroscedastic dependent Berkson errors may occur in the data from a designed experiment. To expand the investigation, the impacts of ignoring the errors in the analysis will be examined for both linear and nonlinear response models. We introduce the problems of mixture experiments with errors, and errors in concentrations from bioassay studies, as examples of heteroscedastic correlated Berkson errors in linear and nonlinear models, respectively.

We also propose new correction approaches. These approaches are obtained by modifying the RC and SIMEX methods to handle Berkson EIV, which is the core novelty in this thesis. We could have looked into some of the methods used to correct Berkson errors, but the novelty of this work relies on the fact that we take methods that have mainly been applied to the classical error model and apply them to Berkson EIV instead.

The challenges we faced in modifying these approaches were quite high. The RC approach not usually used when there are Berkson errors in the data. Carroll et al. (2006) pointed out that using the RC approach with Berkson errors could produce biased estimates of the coefficients, as the Berkson errors may lead to high heteroscedasticity in the observations. In the case of heterogeneity, it is always recommended to use an approach based on the weighted least squares method. However, estimating the weights is rarely straightforward.

The SIMEX approach on the other hand, is not applicable when there are Berkson errors in the data. SIMEX works by adding more errors to the observed variables until a relationship is developed between the error variances and the naive estimates. The observed variables contain measurement errors, and the naive estimates of their coefficients are biased. Thus,

adding errors with increasing variances leads to increasingly biased estimates. This relationship is then used to extrapolate back to the no EIV case, or the actual analysis. This is not the situation in Berkson error models since the actual analysis is the one containing the errors, and the observed variable (mostly the design points) is error free. Hence, adding errors with increasing variances will not guarantee that increasingly biased estimators will be obtained.

The SIMEX approach can however be applied under assumptions of heteroscedastic and correlated classical errors (see for example Wang et al., 1998). Thus, we modify the approach to accept the Berkson error case. Simulation examples are established to examine the effects of these errors on the statistical analysis of EIV models, and to correct for any bias or inefficiency found in the analysis of data.

3 Berkson Errors in Mixture Experiments

3.1 Introduction

In mixture experiments the properties of mixtures are usually studied by mixing the amounts of the mixture components that are required to obtain the necessary proportions. In practice many products produced by mixing several components depend on their proportions in the mixture, but not on the amount of the mixture. Typical examples are the taste of a blend of juices obtained from different fruits and the strength of an alloy made by mixing different metals.

There are many scenarios of mixture experiments involving not only the proportions but also the total amounts of the components in the mixture. These types of experiments are called mixture/amounts experiments. Another type is called process variables mixture experiment, wherein in addition to the proportions, it involves a set of variables called the process variables. These variables have indirect effects on the properties of the mixture, such as, the temperature in which the experiment where established. So, they should be considered in the experiment in order to raise the quality of the mixture.

Although EIV have been widely examined, only few who addressed their effects on mixtures. Fuller (1987, p.79) identified the mixing errors as an interesting problem of errors-

in-variables, but their study appears incomplete. Steiner and Hamada (1997) studied the making of mixtures that are robust to noise and mixing errors. They give an interesting example of how appropriate choice of the setting process variables can ensure good quality of the mixture product. The quality of a product is often defined by the desired value of the response variable (also called *signal*) and the variability of the manufacturing process which is required to be small. Hence a large signal to noise ratio is indicating a good product. However, they do not discuss how mixing errors in the manufacturing of the mixture can be handled. Hamada et al. (2005) pointed out that ignoring the mixing errors leads to results that overestimate the variance of the response of interest, and hence leads to increased confidence intervals for the estimates of the model parameters and reduced power of the statistical tests for their significance. They proposed a Bayesian approach to estimate the parameters of the required statistical models for the data, based on MCMC simulations using prior information about the joint distributions of all model parameters. This empirical approach provides a useful practical tool for a better statistical analysis than that ignoring the mixing errors, but does not allow for establishing all important features of the impact of these errors. They also suggested that the distribution of the actual concentrations could be following a Dirichlet distribution (Connor & Mosimann, 1969), since the proportions of the mixture are no longer fixed design points.

What makes the mixing errors problem difficult is that in designed mixture experiments, errors made in setting the correct amounts of any ingredient propagates to the proportions required by the experimental design for all ingredients. Therefore, the mixing errors lead to a complex error structure for the proportions of the mixtures.

In this chapter we investigate the impact of inaccuracies in discharging the required amounts of the mixture components on the statistical analysis of the data. It is shown that

model (2.3) does not hold when Berkson errors occur in mixture experiments. We then suggest improvements to the naive analysis that ignores the mixing errors.

Sections 3.2 and 3.3 review the main features of data collected in mixture experiments and they show how mixing errors affect the results if they are ignored. Steiner and Hamada (1997) defined a loss function due to the mixing errors but failed to find a closed form for the model bias that occurs. We derive it for the most common standard models under the assumption that reliable estimates for the variances of the mixing errors are available. As simplex lattice designs are frequently used in experiments with mixtures, we also derive the bias in the estimates of the model parameters resulting from statistical analysis of the data that ignores the mixing errors when such designs are used. Then we show how the results of Section 3.3 can be used to eliminate this bias. A method based on the regression calibration approach is used. Examples when the model bias is evaluated analytically, as well as when it is evaluated numerically, are presented and the two approaches are compared. We show that if mixing errors are unavoidable in the manufacturing of a mixture, the quality characteristics will differ on average from the required one. We also use the results of Section 3.3 to choose manufacturing settings so that the bias, the variability of the response, are minimized, thus increasing the signal to noise ratio and the quality of the product. The chapter concludes with a summary and discussion about the usefulness of the presented results.

3.2 Mixture Experiment

In a typical study of a mixture with q components, the experimenter is interested in the way a response of interest, say y, depends on the proportions of the components of the mixture $w_i,\ i = 1,2,\ldots,q$. Hence,

$$0 \le l_i \le w_i \le h_i \le 1,\ \sum_{i=1}^{q} w_i = 1, \tag{3.1}$$

where l_i and h_i are lower and upper bounds for the proportion of the *i*th component in the mixture. A mixture with the desired proportions is obtained by mixing appropriate amounts $W_i,\ i = 1,2,\ldots,q$, of the ingredients. In the absence of errors in setting the amounts of the mixture components, $w_i = W_i / T$, where $T = \sum_{i=1}^{q} W_i$ is the total amount of the mixture. When $l_i = 0$ and $h_i = 1,\ i = 1,2,\ldots,q$, the design region is a regular simplex, which has *q* vertices. Often $l_i > 0$ and $h_i < 1$ for some or all of the mixture components as a result of scientific or practical considerations. If the resulting design region again has *q* vertices, new variables which are linear combinations of the mixture components, called pseudocomponents, can be defined in such a way that the relationships (3.1) are satisfied for these variables. A comprehensive review of results related to experiments with mixtures is given by Cornell (2002).

We will be concerned with the cases wherein the model that describes how a response variable *y* depends on the proportions of the components is linear in the parameters, that is it can be written as

$$\mathbf{y} = \mathbf{F}\boldsymbol{\beta} + \boldsymbol{\varepsilon}, \tag{3.2}$$

where $\mathbf{y}$ and $\boldsymbol{\varepsilon}$ are $n \times 1$ vectors of the responses and the measurement errors, respectively, $\boldsymbol{\beta}$ is a $p \times 1$ vector of the model parameters, while $\mathbf{F}$ is an $n \times p$ extended design matrix, whose *t*th row consists of the values of the regressors of the model evaluated for the *t*th mixture $\mathbf{w}_t = (w_{1t}, w_{2t}, ..., w_{qt})$. We also assume that the errors $\boldsymbol{\varepsilon}$ are independent, normally distributed with zero mean and variance σ_ε^2. An unbiased least squares estimates for (3.2) can be derived by $\hat{\boldsymbol{\beta}} = \left(\mathbf{F}^{\mathbf{T}}\mathbf{F}\right)^{-1}\mathbf{F}^{\mathbf{T}}\mathbf{y}$. The variances of the estimates can be obtained by $\operatorname{var}\left(\hat{\boldsymbol{\beta}}\right) = \left(\mathbf{F}^{\mathbf{T}}\mathbf{F}\right)^{-1}\sigma_\varepsilon^2$.

Standard polynomials cannot be used as models because of the constraints (3.1). Instead, often the canonical polynomials proposed by Scheffé (1958) are used. For example, the first and the second-order Scheffé polynomials are

$$y_t = \sum_{i=1}^{q} \beta_i w_{it} + \varepsilon_t , \tag{3.3}$$

$$y_t = \sum_{i=1}^{q} \beta_i w_{it} + \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \beta_{ij} w_{it} w_{jt} + \varepsilon_t , \tag{3.4}$$

respectively. Other useful models include those proposed by Becker (1968), for example, the models

$$y_t = \sum_{i=1}^{q} \beta_i w_{it} + \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \beta_{ij} \min\left(w_{it}, w_{jt}\right) + \ldots + \beta_{12\ldots q} \min\left(w_{1t}, w_{2t}, \ldots, w_{qt}\right) + \varepsilon_t , \tag{3.5}$$

$$y_t = \sum_{i=1}^{q} \beta_i w_{it} + \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \beta_{ij} \frac{w_{it} w_{jt}}{(w_{it} + w_{jt})} + \beta_{12\ldots q} \frac{w_{1t} w_{2t} \ldots w_{qt}}{(w_{1t} + \ldots + w_{qt})^{q-1}} + \varepsilon_t , \tag{3.6}$$

can be useful when one of the mixture components has an additive blending effect.

Simplex lattice designs (SLD) are often used in mixture experiments when there are no constraints on the mixture components. In a SLD, the proportions of each component take d+1 equally spaced values from 0 to 1, w_i = 0, 1/d, 2/d, ... , 1, for i = 1, 2, ... , q. The analysis of the data obtained with such a design and its interpretation is simple. For example, if d is chosen to be equal to the order of the Scheffé polynomial that will be fitted, the least squares estimators for the model parameters of the first and the second-order Scheffé polynomials are

$$\hat{\beta}_i = \bar{y}_i , \qquad i = 1, \ldots, q , \tag{3.7}$$

and

$$\hat{\beta}_{ij} = 4\bar{y}_{ij} - 2[\bar{y}_i + \bar{y}_j] , \quad i, j = 1, \ldots, q , \quad i<j, \tag{3.8}$$

where $\bar{y}_i$ denotes the average of the observations when the mixture consists of the ith component only, while $\bar{y}_{ij}$ denotes the average of the observations where the proportions of the mixture of the ith and the jth component are both equal to 0.5. When the design region is irregular, the design construction can be carried out using any of the design algorithms described in Atkinson et al. (2007, chap. 12).

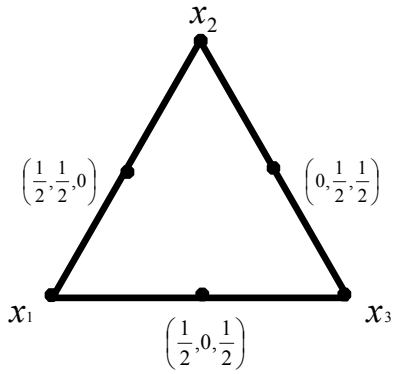
For example, let us consider the case of a SLD with 3-component. If model (3.4) is used to fit the data, the design points of such an experiment can be shown in Table (3.1). The notations y_i and y_{ij}, $i, j = 1, \ldots, q$, $i \neq j$, represent the responses results from pure and binary components mixtures, respectively.

Table 3.1 Design points of a 3-component lattice design

	Proportions			
Trials	w_1	w_2	w_3	Responses
1	1	0	0	y_1
2	0	1	0	y_2
3	0	0	1	y_3
4	0.5	0.5	0	y_{12}
5	0.5	0	0.5	y_{13}
6	0	0.5	0.5	y_{23}

The design region for a mixture experiment of 3-component mixture is usually represented graphically by using an equilateral triangular as in Figure (3.2). In general, for q components, the design region is a q-1 dimensional simplex. This representation is useful to show the design points since each coordinate represents a certain trial, that is one of the formulations of the q-component in the mixture. The maximum values that each component could take are located in the vertices of the triangular, and the points located in the middle of the edges indicate the binary blends.

Figure 3.1 Plot of {3, 2} simplex lattice design.



If one chooses to fit the model using the pseudocomponents, the model will be given by

$$y_t = \sum_{i=1}^{q} \beta_i w'_{it} + \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \beta_{ij} w'_{it} w'_{jt} + \varepsilon_t .$$

where w'_i, $i = 1, \ldots, q$ are pseudocomponents. These components are mainly used to facilitate the analysis of the design under the use of new design points or proportions as a result of the constrained region. For example, if only lower bounds constraints have been imposed on the proportions, the pseudocomponents are computed using the equation

$$w'_i = \frac{w_i - L_i}{1 - L},$$

where $L = \sum_{i=1}^{q} L_i$, and the original proportions will be given by

$$w_i = L_i + (1 - L) w'_i .$$

To illustrate the use of lower bounds constrains, we consider an example in Cornell (2002, pp. 141-143). Assume the following constraints were imposed on the three components $0 \le 0.35 \le w_1 \le 1, 0 \le 0.2 \le w_2 \le 1$, and $0 \le 0.15 \le w_3 \le 1$. Then, the fitted model using the pseudocomponents is

$$y_t = \beta_1 w'_{1t} + \beta_2 w'_{2t} + \beta_3 w'_{3t} + \beta_{12} w'_{1t} w'_{2t} + \beta_{13} w'_{1t} w'_{3t} + \beta_{23} w'_{2t} w'_{3t} + \varepsilon_t ,$$

and by substituting w'_i in y_t, we can write the model in terms of the original components by

$$y_t = \delta_1 w_{1t} + \delta_2 w_{2t} + \delta_3 w_{3t} + \delta_{12} w_{1t} w_{2t} + \delta_{13} w_{1t} w_{3t} + \delta_{23} w_{2t} w_{3t} + \varepsilon_t ,$$

where

$$\delta_1 = \frac{\beta_{12} L_2 (L_1 - 1) + \beta_{13} L_3 (L_1 - 1) + \beta_{23} L_2 L_3}{(1 - L)^2} + \frac{\beta_1 - \sum_{i=1}^{3} \beta_i L_i}{1 - L},$$

$$\delta_2 = \frac{\beta_{12} L_1 (L_2 - 1) + \beta_{13} L_1 L_3 + \beta_{23} L_3 (L_2 - 1)}{(1 - L)^2} + \frac{\beta_2 - \sum_{i=1}^{3} \beta_i L_i}{1 - L},$$

$$\delta_3 = \frac{\beta_{12} L_1 L_2 + \beta_{13} L_1 (L_3 - 1) + \beta_{23} L_2 (L_3 - 1)}{(1 - L)^2} + \frac{\beta_3 - \sum_{i=1}^{3} \beta_i L_i}{1 - L},$$

and

$$\delta_{ij} = \frac{\beta_{ij}}{(1-L)^2}, \qquad i, j = 1,2,3 \ , \quad i < j \ .$$

The following table shows the design points of the above design (Cornell, 2002, p. 143).

Table 3.2 The design points in case of constrained design region of {3,2} lattice design.

	Pseudocomponents			Original proportions			Amounts of components			
Trials	w_1	w_2	w_3	w_1	w_2	w_3	W_1	W_2	W_3	Responses
1	1	0	0	0.65	0.20	0.15	6.5	2	1.5	y_1
2	0	1	0	0.35	0.50	0.15	3.5	5	1.5	y_2
3	0	0	1	0.35	0.20	0.45	3.5	2	4.5	y_3
4	0.5	0.5	0	0.50	0.35	0.15	5	3.5	1.5	y_{12}
5	0.5	0	0.5	0.50	0.20	0.30	5	2	3	y_{13}
6	0	0.5	0.5	0.35	0.35	0.30	3.5	3.5	3	y_{23}

3.3 EIV in Mixture Experiment

When the amounts of the mixture components are set with errors, the actual amount of the *i*th mixture component for the *t*th observation of the response become $X_{it} = W_{it} + e_{it}$, where e_{it} are the errors of setting the amount. We assume that e_{it}, for $i = 1,2,\ldots,q$ and $t = 1,2,\ldots,n$, are independently and normally distributed random variables with mean zero and variance σ_i^2, $i = 1,2,\ldots,q$. The variance of e_{it} is proportional to the discharged amounts, thus the variance of the actual amount X_{it} can be given by $\varphi_i(W_{it})\sigma_i^2$, where $\varphi_i(W_{it})$ defines the way the variance changes with the amount. It could be more interpretive to write the EIV model by $X_{it} = W_{it} + [\varphi_i(W_{it})]^{1/2} e_{it}$, however, to simplify the analytical derivations we achieve later on, we write the model, omitting the part $[\varphi_i(W_{it})]^{1/2}$, and we only make an assumption about the variance of the actual amount. Either way the results are the same, since both writing approaches produces the same inferences for X_{it}.

If only the amount of the *i*th component in the *t*th observation is set with error, the actual proportion of this component becomes $x_{it} = (W_{it} + e_{it})(T + e_{it})^{-1}$, $i = 1,2,...,q$, while the actual proportions of the remaining components become $x_{jt} = W_{jt}(T + e_{it})^{-1}$, $j = 1,2,...,q$, $j \neq i$. We denote the vector of actual proportions $\mathbf{x}_t = (x_{1t}, x_{2t},..., x_{qt})$. If the amounts of all mixture components are set with errors

$$x_{it} = \frac{W_{it} + e_{it}}{T + \sum_{k=1}^{q} e_{kt}}, \quad i = 1,2,...,q.$$

The error in the proportion of the *i*th component resulting from mixing errors in all components of the mixture is

$$u_{it} = \frac{W_{it} + e_{it}}{T'} - \frac{W_{it}}{T} = w_{it} T'^{-1} \left[W_{it}^{-1} e_{it} - T^{-1} \sum_{k=1}^{q} e_{kt} \right],$$

where $T' = T + \sum_{k=1}^{q} e_{kt}$ is the actual total mixture amount.

Clearly, if the mixture consists of a single component, as required for some of the observations of a simplex lattice design, and with no constraints on the proportions of the components, discharging the wrong amount will not change the required proportion of this component, i.e. 1. Therefore the following results are concerned only for cases where the mixtures consist of two or more components, all set with mixing errors. Note that, this argument does not apply if the required proportions are defined in pseudocomponents.

3.4 Effect of Mixing Errors on Mixture Experiments

The impact of the mixing errors on the analysis of the data is summarized below. The results of Lemmas 3.1 and 3.2 are obtained directly by using the delta method. A review on the delta approach is given in Appendix (A).

Lemma 3.1 *The expectation and the variance of the actual proportion of the ith component of a mixture in the tth observation, given the target proportion* w_{it} *are*

$$E\left[x_{it} \mid w_{it}\right] \approx w_{it} + \left(w_{it} - 1\right) T^{-2} \varphi_i \left(W_{it}\right) \sigma_i^2 + w_{it} T^{-2} \sum_{j \neq i}^{q} \varphi_j \left(W_{jt}\right) \sigma_j^2 ,$$

$$\operatorname{var}\left(x_{it} \mid w_{it}\right) \approx \left(1 - w_{it}\right)^2 T^{-2} \varphi_i \left(W_{it}\right) \sigma_i^2 + w_{it}^2 T^{-2} \sum_{j \neq i}^{q} \varphi_j \left(W_{jt}\right) \sigma_j^2 ,$$

$i, j = 1, 2, \ldots, q$, $t = 1, 2, \ldots, n$, *respectively.*

Lemma 3.2 *The expectation and the variance of the product of the actual proportions* x_{it} *and* x_{jt} *of the ith and the jth components of a mixture in the tth observation, given the target proportions* w_{it} *and* w_{jt}*, are*

$$E[x_{it} x_{jt} \mid w_{it}, w_{jt}] \approx w_{it} w_{jt} + (3 w_{it} w_{jt} - 2 w_{jt}) T^{-2} \varphi_i (W_{it}) \sigma_i{}^2 + (3 w_{it} w_{jt} - 2 w_{it}) T^{-2} \varphi_j (W_{jt}) \sigma_j{}^2$$
$$+ 3 w_{it} w_{jt} T^{-2} \sum_{l \neq i \neq j}^{q} \varphi_l (W_{lt}) \sigma_l^2 ,$$

$$\operatorname{var}(x_{it} x_{jt} \mid w_{it}, w_{jt}) \approx w_{jt}^2 (1 - 2 w_{it})^2 T^{-2} \varphi_i (W_{it}) \sigma_i{}^2 + w_{it}^2 (1 - 2 w_{jt})^2 T^{-2} \varphi_j (W_{jt}) \sigma_j{}^2$$
$$+ 4 w_{it}^2 w_{jt}^2 T^{-2} \sum_{l \neq i \neq j}^{q} \varphi_l (W_{lt}) \sigma_l^2 ,$$

$i, j = 1, \ldots, q$, $i < j$, $t = 1, 2, \ldots, n$, *respectively.*

As mentioned before, the actual proportions are correlated. Hence, covariance terms can be obtained easily from Lemma 3.1.

Proof of Lemma 3.1 and Lemma 3.2: The actual *i*th proportion $x_{it} = T'^{-1}(W_{it} + e_{it})$, $i = 1, 2, \ldots, q$, $t = 1, 2, \ldots, n$, where $T' = T + \sum_{k=1}^{q} e_{kt}$, is a function in more than one independent and normally distributed random variables with mean zero and standard deviation σ_i , assumed to be proportional to functions in W_{it} , that is we can write $e_{it} \sim N(0, \varphi(W_{it}) \sigma_i^2)$, e_{it} is the error in the *i*th component. Let z_t is the sum of the errors in all the components in the *t*th trial, except the *i*th one $z_t = \sum_{j \neq i}^{q} e_{jt}$, which follows a normal distribution with mean zero and variance $\sum_{j \neq i}^{q} \phi(W_{jt}) \sigma_j^2$. By using approximation (A.1 in Appendix A), the second-order series expansion of u_{it} around $e_{it} = 0$ and $z_t = 0$ is given by

$$u_{it} \approx g(\mu_{e_i}, \mu_z) + (e_{it} - \mu_i) g'_{e_i}(\mu_{e_i}, \mu_z) + (z - \mu_z) g'_z(\mu_{e_i}, \mu_z)$$

$$+ \frac{1}{2}(e_i - \mu_{e_i})^2 g''_{e_i}(\mu_{e_i}, \mu_z) + \frac{1}{2}(z - \mu_z)^2 g''_z(\mu_{e_i}, \mu_z)$$

where

$$g'_{e_i}(\mu_{e_i}, \mu_z) = \frac{\partial g}{\partial e_i} = (T - W_{it})(T + e_{it} + z)^{-1} T^{-1} - (Te_{it} - W_{it}e_{it} - W_{it}z_t)(T + e_{it} + z_t)^{-2} T^{-1}$$

$$g''_{e_i}(\mu_{e_i}, \mu_z) = \frac{\partial^2 g}{\partial e_i \partial e_i} = -2(T - W_{it})(T + e_{it} + z_t)^{-2} T^{-1} + 2(Te_{it} - W_{it}e_{it} - W_{it}z_t)(T + e_{it} + z_t)^{-3} T^{-1}$$

$$g'_z(\mu_{e_i}, \mu_z) = \frac{\partial g}{\partial z} = -W_{it}(T + e_{it} + z_t)^{-1} T^{-1} - (Te_{it} - W_{it}e_{it} - W_{it}z_t)(T + e_{it} + z_t)^{-2} T^{-1}$$

$$g''_z(\mu_{e_i}, \mu_z) = \frac{\partial^2 g}{\partial z \partial z} = 2W_{it}(T + e_{it} + z_t)^{-2} T^{-1} + 2(Te_{it} - W_{it}e_{it} - W_{it}z_t)(T + e_{it} + z_t)^{-3} T^{-1},$$

and from $x_{it}=w_{it}+u_{it}$, a second-order Taylor series expansion of x_{it} around $e_{it} = 0$ and $z_t = 0$ is $x_{it} \approx w_{it} + (1 - w_{it})T^{-1} e_{it} - w_{it} T^{-1} z_t - (1 - w_{it})T^{-2} e_{it}^2 + w_{it} T^{-2} z_t^2$. Hence,

$$E\left[x_{it} \mid w_{it}\right] \approx w_{it} + \left(w_{it} - 1\right) T^{-2} \varphi_i\left(W_{it}\right) \sigma_i^2 + w_{it} T^{-2} \sum_{j \neq i}^{q} \varphi_j\left(W_{jt}\right) \sigma_j^2, \quad i, j = 1, 2, \ldots, q.$$

Now by taking the variance of the Taylor series expansion of x_{it}, the following terms will appear, $\operatorname{cov}(e_i, e_i^2)$, $\operatorname{var}(e_i^2)$, $\operatorname{cov}(z_t, z_t^2)$, and $\operatorname{var}(z_t^2)$, $i = 1, \ldots, q$, $t = 1,2, \ldots, n$.

The error components are assumed to be independent and normally distributed with zero means and different variances, hence $\operatorname{cov}(e_i, e_i^2) = 0$, $\operatorname{var}(e_i^2) = 2\sigma_i^4$ and $\operatorname{cov}(z_t, z_t^2) = 0$. Also

$z_t^2 = \left(\sum_{j \neq i}^{q} e_{jt} \right)^2 = \sum_{j \neq i}^{q} e_{jt}^2 + 2 \sum_{j \neq i}^{q-1} \sum_{l=j+1}^{q} e_{jt} e_{lt}$, where $l \neq i$ and $t = 1,2, \ldots, n$. So,

$$\operatorname{var}(z_t^2) = \operatorname{var}\left(\sum_{j \neq i}^{q} e_{jt}^2 \right) + 4 \operatorname{var}\left(\sum_{j \neq i}^{q-1} \sum_{l=j+1}^{q} e_{jt} e_{lt} \right) + 2 \operatorname{cov}\left(\sum_{j \neq i}^{q} e_{jt}^2, \sum_{j \neq i}^{q-1} \sum_{l=j+1}^{q} e_{jt} e_{lt} \right).$$

Direct calculations show that $\operatorname{var}(z_t^2) = 2 \sum_{j \neq i}^{q} \sigma_j^4$, $t = 1,2, \ldots, n$. Since $\operatorname{var}(e_i^2)$ and $\operatorname{var}(z_t^2)$ are too small to matter

$$\operatorname{var}\left(x_{it} \mid w_{it}\right) \approx \left(1 - w_{it}\right)^2 T^{-2} \varphi_i\left(W_{it}\right) \sigma_i^2 + w_{it}^2 T^{-2} \sum_{j \neq i}^{q} \varphi_j\left(W_{jt}\right) \sigma_j^2, \quad i, j = 1, 2, \ldots, q,$$

$t = 1,2, \ldots, n$.

Similar to proof of lemma 3.1, applying delta method for

$$x_{it}x_{jt} = (w_{it}T + e_{it})(w_{jt}T + e_{jt})(T + e_{it} + e_{jt} + z_t)^{-2},\ i, j = 1,\ldots,q,\ \ i < j,$$

gives

$$E[x_{it}x_{jt} \mid w_{it}, w_{jt}] \approx w_{it}w_{jt} + (3w_{it}w_{jt} - 2w_{jt})T^{-2}\varphi_i(W_{it})\sigma_i{}^2 + (3w_{it}w_{jt} - 2w_{it})T^{-2}\varphi_j(W_{jt})\sigma_j{}^2$$
$$+3w_{it}w_{jt}T^{-2}\sum_{l\neq i\neq j}^{q}\varphi_l(W_{lt})\sigma_l^2,\quad i, j, l = 1,2,\ldots,q,\ \ i < j,\quad t = 1,2,\ldots,n.$$

and

$$\mathrm{var}(x_{it}x_{jt} \mid w_{it}, w_{jt}) \approx w_{jt}^2(1-2w_{it})^2T^{-2}\varphi_i(W_{it})\sigma_i{}^2 + w_{it}^2(1-2w_{jt})^2T^{-2}\varphi_j(W_{jt})\sigma_j{}^2$$
$$+4w_{it}^2w_{jt}^2T^{-2}\sum_{l\neq i\neq j}^{q}\varphi_l(W_{lt})\sigma_l^2,\quad i, j, l = 1,2,\ldots,q,\ \ i < j,\quad t = 1,2,\ldots,n.$$

Special Case: Lemmas 3.1 and 3.2 show that, the means of both the actual proportions and their products are different from those specified by the experimental design, and their variances increase with the values of the EIV variances. For example, in a SLD, if mixing errors occur in the amounts of the components of a binary blend where $w_{it} = w_{jt} = 0.5$,

$$E[x_{it} \mid w_{it}] \approx 0.5 - 0.5T^{-2}\left[\varphi_i(W_{it})\sigma_i^2 - \varphi_j(W_{jt})\sigma_j^2\right],$$

and

$$\mathrm{var}(x_{it} \mid w_{it}) \approx 0.25T^{-2}\left[\varphi_i(W_{it})\sigma_i^2 + \sum_{j\neq i}^{q}\varphi_j(W_{jt})\sigma_j^2\right].$$

An important special case is when $\sigma_i^2 = \sigma_j^2$ and $\varphi_i = \varphi_j$, as then $E[x_{it} \mid w_{it}] \approx w_{it}$, which means that the target proportions are achieved on average, with an increased variability. However, even in this situation the use of second or higher order polynomial models is affected. For example,

$$E\left[x_{it}x_{jt} \mid w_{it}, w_{jt}\right] \approx 0.25\left[1 - 2T^{-2}\varphi_i(W_{it})\sigma_i{}^2\right],$$

is less then the regressor $w_{it}w_{jt} = 0.25$, specified by the experimental design.

Theorem 3.1 *The mixing errors make the model biased, except in the case when the mixture consists of a single component, that is*

$$E[y_t \mid \mathbf{w}_t] = \eta(\boldsymbol{\beta}, \mathbf{w}_t) + B, \qquad t = 1,2,\ldots,n,$$

where $\eta(\boldsymbol{\beta}, \mathbf{w}_t)$ *is the true response for the mixture* $\mathbf{w}_t$*, and the bias B depends on the form of the true model.*

The exact impact of the mixing errors on the statistical analysis of the results depends on the experimental design that has been used and the statistical model that is estimated. As illustration of this theorem, the derivations of the biases of the first and second-order Scheffé polynomial models is given as follows.

Proof of Theorem 3.1 for the First-Order Scheffé Polynomial Model: The result of this theorem follows directly from Lemmas 3.1 and 3.2. If the amounts of all mixture components are discharged with errors, for $t = 1,2,\ldots,n$, the true model is $y_t = \sum_{i=1}^{q} \beta_i x_{it} + \varepsilon_t$. From Lemma 3.1, it follows that a naive analysis of the data ignoring the mixing errors using model (3.3) leads to predictions

$$E\left[y_t \mid \mathbf{w}_t\right] \approx \sum_{i=1}^{q} \beta_i w_{it} + T^{-2} \sum_{i=1}^{q} \beta_i \left[\left(w_{it} - 1\right) \varphi_i \left(W_{it}\right) \sigma_i^2 + w_{it} \sum_{j \neq i}^{q} \varphi_j \left(W_{jt}\right) \sigma_j^2 \right] = \eta\left(\boldsymbol{\beta}, \mathbf{w}_t\right) + B$$

where $B = T^{-2} \sum_{i=1}^{q} \beta_i \left[\left(w_{it} - 1\right) \varphi_i \left(W_{it}\right) \sigma_i^2 + w_{it} \sum_{j \neq i}^{q} \varphi_j \left(W_{jt}\right) \sigma_j^2 \right]$. Hence, the expected response is biased.

Proof of Theorem 1 for the Second-Order Scheffé Polynomial Model: If one or more of the mixture components are discharged with errors, the true model for $t = 1,2,\ldots,n$, is given by $y_t = \sum_{i=1}^{q} \beta_i x_{it} + \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \beta_{ij} x_{it} x_{jt} + \varepsilon_t$. It follows from Lemmas 3.1 and 3.2 that

$$E[y_t \mid \mathbf{w}_t] \approx \sum_{i=1}^{q} \beta_i w_{it} + \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \beta_{ij} w_{it} w_{jt} + B = \eta(\boldsymbol{\beta}, \mathbf{w}_t) + B, \tag{3.9}$$

where the bias is

$$B = T^{-2} \sum_{i=1}^{q} \beta_i \left[(w_{it} - 1) \varphi_i (W_{it}) \sigma_i^2 + w_{it} \sum_{j \neq i}^{q} \varphi_j (W_{jt}) \sigma_j^2 \right] + T^{-2} \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \beta_{ij} \left[(3 w_{it} w_{jt} - 2 w_{jt}) \varphi_i (W_{it}) \sigma_i^2 \right.$$

$$+(3w_{it}w_{jt}-2w_{it})\varphi_j(W_{jt})\sigma_j{}^2+3w_{it}w_{jt}\sum_{l\neq i\neq j}^{q}\varphi_l(W_{lt})\sigma_l^2\Bigg].$$

Theorem 3.2 *The mixing errors make the variance of the model heterogeneous and larger than* σ_ε^2, *i.e.* $\text{var}(y_t \mid \mathbf{w}_t) > \sigma_\varepsilon^2$.

The expression for the variance of the response depends on the true model and can be very complex. Therefore we only show its derivation for the first-order Scheffé polynomial model.

Proof of Theorem 2 for the First-Order Scheffé Polynomial Model: For $t = 1,2,\ldots,n$, the variance of the response given the design points is

$$\text{var}(y_t \mid \mathbf{w}_t) \approx \sigma_\varepsilon^2 + \sum_{i=1}^{q}\beta_i^2\,\text{var}(x_{it} \mid w_{it}) + 2\sum_{i=1}^{q-1}\sum_{j=i+1}^{q}\beta_i\beta_j\,\text{cov}(x_{it}, x_{jt}).$$

Since $\text{cov}(x_i, x_j) = E[x_i x_j] - E[x_i]E[x_j]$, $i, j = 1,\ldots,q$, $i < j$, applying the results of Lemmas 3.1 and 3.2, gives

$$\begin{aligned}
\text{var}(y_t \mid \mathbf{w}_t) \approx \sigma_\varepsilon^2 &+ T^{-2}\sum_{i=1}^{q}\beta_i^2\Bigg[(w_{it}-1)^2\varphi_i(W_{it})\sigma_i^2 + w_{it}^2\sum_{j\neq i}^{q}\varphi_j(W_{jt})\sigma_j^2\Bigg] \\
&+2\sum_{i=1}^{q}\sum_{j=i+1}^{q-1}\beta_i\beta_j\Bigg\{\Big[w_{it}w_{jt}+(3w_{it}w_{jt}-2w_{jt})T^{-2}\varphi_i(W_{it})\sigma_i{}^2 \\
&+(3w_{it}w_{jt}-2w_{it})T^{-2}\varphi_j(W_{jt})\sigma_j{}^2+3w_{it}w_{jt}T^{-2}\sum_{l\neq i\neq j}^{q}\varphi_l(W_{lt})\sigma_l^2\Big] \\
&-\Bigg[w_{it}+T^{-2}(w_{it}-1)\varphi_i(W_{it})\sigma_i^2 + w_{it}T^{-2}\sum_{l\neq i}^{q}\varphi_l(W_{lt})\sigma_l^2\Bigg] \\
&\times\Bigg[w_{jt}+(w_{jt}-1)T^{-2}\varphi_j(W_{jt})\sigma_j^2 + w_{jt}T^{-2}\sum_{l\neq j}^{q}\varphi_l(W_{lt})\sigma_l^2\Bigg]\Bigg\}. \qquad (3.10)
\end{aligned}$$

Clearly this variance is larger than σ_ε^2 and depends on the proportions of the mixture components and the variances of the mixing errors.

Theorem 3.3 *If a simplex lattice design, with d = 2, is used, the expectations of the least squares estimators for the parameters of models (3.3) and (3.4) are*

$$E[\hat{\beta}_i] = \beta_i,\ E[\hat{\beta}_{ij}] \approx \beta_{ij} - B_{ij},\quad i, j = 1,\ldots,q,\ \ i < j,$$

where $B_{ij} = \left[\varphi_i(W_{it})\sigma_i^{\,2} + \varphi_j(W_{jt})\sigma_j^{\,2}\right] T^{-2}\beta_{ij} + 2\left[\varphi_i(W_{it})\sigma_i^{\,2} - \varphi_j(W_{jt})\sigma_j^{\,2}\right] T^{-2}(\beta_i - \beta_j)$,

$t = 1,2,\ldots,n$. *Hence, the estimates* $\hat{\beta}_i$ *are unbiased for* β_i, *and the estimates* $\hat{\beta}_{ij}$ *are approximately biased for* β_{ij}.

Proof of Theorem 3.3: In simplex lattice designs, since only one or two components mixtures are obtained, we can cancel the part concerned with three or higher components mixture in model (3.9), so that

$$E[y_t \mid \mathbf{w}_t] \approx \sum_{i=1}^{q} \beta_i \left[w_{it} + (w_{it} - 1)T^{-2}\varphi_i(W_{it})\sigma_i^2 + w_{it}T^{-2}\varphi_j(W_{jt})\sigma_j^2 \right] + \sum_{i=1}^{q-1}\sum_{j=i+1}^{q} \beta_{ij}\left[w_{it}w_{jt} + (3w_{it}w_{jt} - 2w_{jt})T^{-2}\varphi_i(W_{it})\sigma_i^{\,2} + (3w_{it}w_{jt} - 2w_{it})T^{-2}\varphi_j(W_{jt})\sigma_j^{\,2}\right]. \tag{3.11}$$

The naive least squares estimates of the parameters of model (3.4) are

$$\hat{\boldsymbol{\beta}}_{naive} = \left(\mathbf{F}_{\mathbf{w}}^{\mathbf{T}}\mathbf{F}_{\mathbf{w}}\right)^{-1}\mathbf{F}_{\mathbf{w}}^{\mathbf{T}}\mathbf{y}\,. \tag{3.12}$$

In simplex lattice designs with six trails, the extended design matrix is given by

$$\mathbf{F}_{\mathbf{w}} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0.5 & 0.5 & 0 & 0.25 & 0 & 0 \\ 0.5 & 0 & 0.5 & 0 & 0.25 & 0 \\ 0 & 0.5 & 0.5 & 0 & 0 & 0.25 \end{bmatrix}.$$

Then,

$$\left(\mathbf{F}_{\mathbf{w}}^{\mathbf{T}}\mathbf{F}_{\mathbf{w}}\right)^{-1}\mathbf{F}_{\mathbf{w}}^{\mathbf{T}} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ -2 & -2 & 0 & 4 & 0 & 0 \\ -2 & 0 & -2 & 0 & 4 & 0 \\ 0 & -2 & -2 & 0 & 0 & 4 \end{bmatrix}.$$

Now, for $i, j = 1,\ldots,q$, $i<j$, it follows from (3.11) that

$$E[y_{ijt} \mid \mathbf{w}_t] \approx \beta_i \left[w_{it} + (w_{it} - 1)T^{-2}\varphi_i(W_{it})\sigma_i^2 + w_{it}T^{-2}\varphi_j(W_{jt})\sigma_j^2 \right]$$
$$+ \beta_j \left[w_{jt} + (w_{jt} - 1)T^{-2}\varphi_j(W_{jt})\sigma_j^2 + w_{jt}T^{-2}\varphi_i(W_{it})\sigma_i^2 \right]$$
$$+ \beta_{ij} \left[w_{it}w_{jt} + (3w_{it}w_{jt} - 2w_{jt})T^{-2}\varphi_i(W_{it})\sigma_i{}^2 + (3w_{it}w_{jt} - 2w_{it})T^{-2}\varphi_j(W_{jt})\sigma_j{}^2 \right], \quad (3.13)$$

and $E[y_i] = \beta_i$, $i = 1, \ldots, q$. Putting $w_i = w_j = 0.5$ in (3.13), and taking the expectation of (3.12) gives $E[\hat{\beta}_i] = \beta_i$, $E[\hat{\beta}_{ij}] \approx \beta_{ij} - B_{ij}$, $i, j = 1, \ldots, q$, $i < j$, where,

$B_{ij} = \left[\varphi_i(W_{it})\sigma_i{}^2 + \varphi_j(W_{jt})\sigma_j{}^2 \right] T^{-2}\beta_{ij} + 2\left[\varphi_i(W_{it})\sigma_i{}^2 - \varphi_j(W_{jt})\sigma_j{}^2 \right] T^{-2}(\beta_i - \beta_j)$. Such results can also be derived for special cases when other models and other experimental designs are used.

3.5 Adjusted Statistical Analysis

The results presented in this section show that if the variances of the mixing errors are known, or can be estimated, the impact of these errors can be minimized. We start with developing a method for estimating the model parameters supported with a simulation example. It shows how the regression calibration approach can be used to minimize the resulting bias in the model and in the estimates of the model parameters, as well as to find correct estimates of the corresponding variances. Its application is made difficult by the complex structure of mixing errors. We also show how knowledge of the form of the model bias allows for choosing a manufacturing setting for a mixture product that is not biased and has a larger signal to noise ratio.

3.5.1 Estimating Unknown Model Parameters

If the form of the bias in the naive estimates is known, the mean bias can be estimated and used to adjust the naive parameter estimates. For example, Theorem 3.3 can be used to show that if a SLD is used, the bias-corrected estimator $\hat{\beta}_{c(ij)}$ for parameters β_{ij} of the second-order Scheffé polynomial is

$$\hat{\beta}_{c(ij)} \approx \frac{\hat{\beta}_{ij} + 2\left[\varphi_i(W_{it})\sigma_i^{\,2} - \varphi_j(W_{jt})\sigma_j^{\,2}\right] T^{-2}\left(\hat{\beta}_i - \hat{\beta}_j\right)}{1 - \left[\varphi_i(W_{it})\sigma_i^{\,2} + \varphi_j(W_{jt})\sigma_j^{\,2}\right] T^{-2}}, \tag{3.14}$$

where $1 - \varphi_i(W_{it})\sigma_i^{\,2}T^{-2} - \varphi_j(W_{jt})\sigma_j^{\,2}\ T^{-2} \neq 0$, $i, j = 1,\ldots,q,\ \ i < j$, $t = 1,2,\ldots,n$.

Clearly, finding the exact form of the bias and its variance can be a cumbersome task, as it depends on the experimental design and the model being used. The result given by equation (3.14) was obtained following the idea of the regression calibration approach. In general, the estimator for the model parameters of model (3.2), corrected for bias caused by mixing errors can be given by $\hat{\boldsymbol{\beta}}_c = \left(\mathbf{F}_c^{\mathrm{T}}\mathbf{F}_c\right)^{-1}\mathbf{F}_c^{\mathrm{T}}\mathbf{y}$, where $\mathbf{F}_c$ is the expectation of the actual extended design matrix $\mathbf{F}_\mathbf{x}$, given the specified by the experimental design extended design matrix $\mathbf{F}_\mathbf{w}$, i.e. $\mathbf{F}_c = E\left[\mathbf{F}_\mathbf{x} \mid \mathbf{F}_\mathbf{w}\right]$. The standard errors of the corrected estimates are the square roots of the diagonal elements of the matrix $\left(\mathbf{F}_c^{\mathrm{T}}\mathbf{F}_c\right)^{-1}\sigma_c^2$, where σ_c^2 is the residuals variance from the regression of y on the estimate of the expectation of the actual design given the target one. If the variances of the mixing errors are small, the difference between $\mathbf{F}_c$ and $\mathbf{F}_\mathbf{w}$ may be too small to matter in practice. However, as σ_i^2, $i = 1,2,\ldots,q$, increases, the mixing errors lead to parameter estimates with larger variance than in the case when no mixing errors have been made. All elements of $\mathbf{F}_c$ can be obtained by using the results of lemmas 3.1 and 3.2. However, when finding such expectations analytically is very difficult, e.g. Becker's models; they can be obtained empirically using computer simulation. We refer to these correction methods as RC_A and RC_E, respectively.

These correction approaches are more effective when the response has homoscedastic variance. If it does not, $\hat{\boldsymbol{\beta}}_c$ is approximately asymptotically unbiased but inefficient. If an estimate of σ_ε^2 is available, efficient estimate of $\hat{\boldsymbol{\beta}}$ can be obtained using the WLS method, as recommended by Carroll et al. (2006). Because of the complexity of the structure of the

mixing errors, the form of the response variance matrix Σ is difficult to derive for second and higher order models and it will be estimated empirically. This can be done based on some information about the mean and variance of the response errors.

Note that, cases wherein Σ is unknown with no EIV, have been extensively studied in the literature by many authors, such as, Fuller and Rao (1978), Cragg (1983), Carroll and Cline (1988), and White (1980). In particular Fuller and Rao (1978) and Carroll and Cline (1988) are of interest here. They suggest obtaining estimates of the weights by taking the standard deviations of replications of the responses at each design value. In their work, restrictions on the appropriate number of replications have been made to insure unbiased estimator of $\hat{\Sigma}$. This approach is always useful to be used if possible, as it provides more information for the problem under study. However, the number of replications should be large enough to provides an appropriate estimate of Σ. We here use an empirical replication approach, so that no restriction on the number of replications is needed. The empirical approach is based on an estimate of the variance σ_{ε}^2 of the regression errors. These estimates are used to generate a large number of replications at each design point, after which Σ is obtained. So unlike their approach, here large number of responses can be obtained without any practical restriction. However, validation data or knowledge from previous experiments is needed.

The proposed correction approach, which we call weighted regression calibration (WRC), is based on three steps. First the RC is applied in order to find initial estimates $\hat{\boldsymbol{\beta}}_c$. Then Σ is estimated using these estimates. Finally, $\hat{\Sigma}$ and $\mathbf{F}_c$ are used to find the weighted least squares estimates of the model parameters given by $\hat{\boldsymbol{\beta}}_c = (\mathbf{F}_c^{\mathbf{T}}\hat{\Sigma}^{-1}\mathbf{F}_c)^{-1}\mathbf{F}_c^{\mathbf{T}}\hat{\Sigma}^{-1}\mathbf{y}$, where $\hat{\boldsymbol{\beta}}_c$ now is approximately asymptotically unbiased and efficient estimator of $\boldsymbol{\beta}$, whose standard errors are given by the square roots of the diagonal elements of the matrix $(\mathbf{F}_c^{\mathbf{T}}\hat{\Sigma}^{-1}\mathbf{F}_c)^{-1}$. When

$\mathbf{F}_c$ is obtained analytically we denote the method WRC_A, while when this is done empirically, we denote it WRC_E.

In order to generalize the results, we choose in the following simulation examples to study the effect of mixing errors using the second-order Scheffé model (3.4). In all the simulations, the models are assumed to be fit with small response variance, in order to make the interpretation for the effect of the mixing errors on the analysis clearer, and to avoid any computational problems.

3.5.2 Response Prediction

Often the objective of mixture experiments is to obtain a statistical model that allows us to predict the response for a variety of mixtures. Predicting the response for a mixture, $\mathbf{w}_a$ say, can be obtained using the corrected parameter estimates $\hat{\boldsymbol{\beta}}_c$, and the prediction $\hat{y}(\mathbf{x}_a) = \hat{\boldsymbol{\beta}}_c^{\mathbf{T}} \mathbf{x}_a$ will be asymptotically unbiased. However, the prediction variance will increase with the variances of the mixing errors.

3.5.3 Simulation Example

Design Choice and Simulation Parameters: We assume that the true model is the second-order Scheffé polynomial (3.4) with $\beta_1 = 250$, $\beta_2 = 175$, $\beta_3 = 190$, $\beta_{12} = 550$, $\beta_{13} = 380$, and $\beta_{23} = 450$. The experimental errors are assumed to be independent and normally distributed with mean zero and common variance 4×10^{-4}. The experimental design that is used is given in Table 3.3. This is the same design that Cornell (2002, p. 297) used to study the effect of powder pesticide in combination with two liquid pesticides to suppress mite population numbers.

Table 3.3 Experimental design (Cornell, 2002, p. 297) for the simulated experiment.

	Proportions		
Run	w_1	w_2	w_3
1	1	0	0
2	0	1	0
3	0	0	1
4	0.50	0.50	0
5	0.50	0	0.50
6	0	0.50	0.50
7	0.20	0.20	0.60
8	0	0.75	0.25
9	0	0.25	0.75
10	0.25	0	0.75
11	0.75	0	0.25
12	0.40	0.40	0.20
13	0.30	0.30	0.40
14	0.25	0.25	0.50
15	0.10	0.10	0.80

In this example, we assume that the experimental design is implemented by mixing certain amounts of the mixture components, as required by the experimental design, so that the total amount of the mixture is one. However, the required amounts of the three mixture components are discharged with errors, assumed to be normally distributed with zero means, and variances $W_{1t}^2\sigma_1^2$, $W_{2t}^2\sigma_2^2$, and $W_{3t}^2\sigma_3^2$, $t = 1,2,\ldots,n$, respectively, where $\sigma_1 = 0.07$, $\sigma_2 = 0.08$, and $\sigma_3 = 0.10$. The matrix $\mathbf{F}_c$ is obtained empirically by averaging the results of 10^4 simulations at each design. An empirical estimate of $\hat{\Sigma}$ is also obtained by using the variances of the responses generated over the same number of simulations at each design point.

In order to compare results based on the analytical and empirical evaluations of $\mathbf{F}_c$, the calibrated design is also obtained analytically. Note that the validity of the simulation codes was tested by using different simulation conditions. For example, if the EIV variances are set to zero, all the approaches of analyses are supposed to have equivalent results. Moreover, by increasing the size of the EIV, a trend of enlarged effects for the EIV was expected in the results.

Simulation Results: Table 3.4 lists the biases of the estimates of the model parameters when the following five methods of estimation are used:

- Actual analysis, where the actual concentrations are known.
- Naive analysis, ignoring the mixing errors.
- Analysis when the correction to the model parameters is based on the analytical and empirical estimates of $\mathbf{F}_c$, ignoring the possibility for heterogeneity in the variances of the responses, RC_A and RC_E, respectively.
- The corresponding analysis when the corrections are obtained using the WRC_A and WRC_E methods, respectively.

Table 3.4 Bias of the estimators of the model parameters obtained using the actual, naive, RC_E, RC_A, WRC_E and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	-2.613×10^{-5}	-4.040×10^{-2}	-7.255×10^{-3}	1.166×10^{-2}	-1.169×10^{-5}	-1.153×10^{-5}
β_2	-4.178×10^{-5}	-5.209×10^{-2}	-4.706×10^{-3}	-1.078×10^{-2}	-4.633×10^{-5}	-4.633×10^{-5}
β_3	-8.214×10^{-5}	0.133	-8.048×10^{-3}	-6.208×10^{-2}	-1.392×10^{-4}	-1.395×10^{-4}
β_{12}	9.214×10^{-5}	-1.619	1.880×10^{-2}	3.730×10^{-2}	-3.768×10^{-3}	6.012×10^{-3}
β_{13}	5.782×10^{-4}	-0.998	-2.818×10^{-2}	-5.371×10^{-2}	3.580×10^{-2}	2.829×10^{-3}
β_{23}	1.470×10^{-4}	-1.113	1.204×10^{-2}	1.611×10^{-2}	-2.293×10^{-2}	-4.663×10^{-2}

The actual analysis shows what the correct estimates would be if the actual mixtures were known. Table 3.4 indicates that the actual estimates are unbiased. The results in Table 3.4 confirm that the naive estimators of the model parameters are asymptotically biased if the mixing errors are ignored. The bias is substantially reduced when the analytical estimate of $\mathbf{F}_c$ is used, but it is virtually eliminated when the empirical estimate of $\mathbf{F}_c$ is used. This result is not surprising because the analytical approach is based on repeated use of the delta method, which provides only approximate values of the elements of $\mathbf{F}_c$. In addition, Table 3.4 indicates that using the modified weighted least squares approach eliminates the bias in the estimates of the main effects.

Table 3.5 gives the averages of the estimated standard errors (denoted by SEs), computed using the square root of the ith diagonal element of the matrix $\left(\mathbf{F}_{\cdot}^{\mathbf{T}}\mathbf{F}_{\cdot}\right)^{-1}\hat{\sigma}^2$, where $\mathbf{F}_{\cdot}$ is either $\mathbf{F}_{\mathbf{x}}$, $\mathbf{F}_{\mathbf{w}}$, or $\mathbf{F}_c$, for the least squares estimates, or $(\mathbf{F}_c^{\mathbf{T}}\hat{\Sigma}^{-1}\mathbf{F}_c)^{-1}$, when the WRC method is used. In addition, the estimates of the SEs are obtained from the simulations or they are Monte Carlo standard errors, computed by $\sqrt{\sum_{j=1}^{10,000}(\hat{\beta}_{ij}-\beta_{ij})^2/9999}$, where i is the subscript of β: 1, 2, 3, 12, 13 or 23.

Table 3.5 The MC SE (the averaged model-based SE) of the estimators of the model parameters obtained using the actual, naive, RC_E, RC_A, WRC_E, and WRC_A, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	9.118×10^{-3} (9.234×10^{-3})	1.360 (4.060)	1.358 (4.059)	1.359 (4.059)	9.960×10^{-3} (9.959×10^{-3})	9.960×10^{-3} (1.000×10^{-2})
β_2	9.117×10^{-3} (9.231×10^{-3})	1.931 (4.060)	1.929 (4.057)	1.929 (4.058)	9.990×10^{-3} (9.989×10^{-3})	9.990×10^{-3} (1.003×10^{-2})
β_3	8.229×10^{-3} (8.217×10^{-3})	2.515 (3.615)	2.507 (3.618)	2.506 (3.619)	1.001×10^{-2} (1.001×10^{-2})	1.001×10^{-2} (9.915×10^{-3})
β_{12}	3.959×10^{-2} (3.989×10^{-2})	10.070 (17.470)	9.978 (17.512)	9.981 (17.515)	7.102 (7.056)	7.104 (7.061)
β_{13}	3.737×10^{-2} (3.787×10^{-2})	10.453 (16.616)	10.441 (16.662)	10.443 (16.664)	6.797 (6.814)	6.796 (6.819)
β_{23}	3.752×10^{-2} (3.786×10^{-2})	10.773 (16.616)	10.756 (16.658)	10.752 (16.656)	3.343 (3.356)	3.343 (3.353)

The results in Table 3.5 show that the mixing errors increase the standard errors of the model parameters. In the naive analysis, the RC_A and RC_E approaches overestimate them and the differences between the three are negligible. The standard errors of the estimates obtained using the WRC_A and WRC_E approaches are considerably reduced, especially those for β_1, β_2 and β_3. Furthermore, they are similar to the standard errors of the model parameters obtained using the actual design.

The correction for the parameters corresponding to the component interactions is less effective, although it is still better than when RC_A and RC_E are used. Their standard errors are

larger than those for the main effects, by design, and this difference is magnified substantially by the mixing errors. Note that, in practice, it would not be possible to compute the true standard errors S_{1i} as the true values of the model parameters would not be known. However, S_{2i} can be computed easily and they also appear to be correct.

To provide an additional interpretation of the effect on the parameter and variance estimates, we compute the percentage of absolute mean relative bias given by $|E[\hat{\boldsymbol{\beta}} - \boldsymbol{\beta}]/\boldsymbol{\beta}| \times 100$. We also compute the relative standard error as a percentage given by [Averaged model-based SE / Monte Carlo SE]. These two measures help us to understand the benefits of applying the correction methods to analyze the results.

Table 3.6 shows the percentage of absolute mean relative bias among the estimates from the actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated using 10^4 Monte Carlo simulations. The results in Table 3.6 show how applying the correction approaches RC_E and RC_A improve the estimates of the parameters. When the WRC_E and WRC_A approaches are used, the biases in the naive estimates are completely eliminated.

Table 3.6 Percentage of absolute mean relative bias of the estimators of the model parameters obtained using the actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	1.045×10^{-5}	1.616×10^{-2}	2.902×10^{-3}	4.664×10^{-3}	4.676×10^{-6}	4.612×10^{-6}
β_2	2.387×10^{-5}	2.977×10^{-2}	2.689×10^{-3}	6.160×10^{-3}	2.647×10^{-5}	2.647×10^{-5}
β_3	4.323×10^{-5}	7.000×10^{-2}	4.236×10^{-3}	3.267×10^{-2}	7.326×10^{-5}	7.342×10^{-5}
β_{12}	1.675×10^{-5}	0.294	3.418×10^{-3}	6.782×10^{-3}	6.851×10^{-4}	1.093×10^{-3}
β_{13}	1.522×10^{-4}	0.263	7.416×10^{-3}	1.413×10^{-2}	9.421×10^{-3}	7.445×10^{-4}
β_{23}	3.267×10^{-5}	0.247	2.676×10^{-3}	3.580×10^{-3}	5.096×10^{-3}	1.036×10^{-2}

Table 3.7 illustrates the efficiency of the actual, naive, RC_E, RC_A, WRC_E, and WRC_A estimators. The relative standard errors from the naive, RC_E and RC_A approaches are seen to be approximately similar, although a slight reduction in the standard errors of the estimators when using RC_E and RC_A can be noticed in Table 3.4 but this difference is probably insignificant. Therefore, using these approaches leads to approximately unbiased but

inefficient estimates. The gain in efficiency when using both the WRC_E and WRC_A approaches is considerably high. In fact, WRC_E gives an efficiency of approximately 1 for the estimates of the pure components. Estimates of the binary components are also efficiently high, with ratios of 0.994, 1.003, and 1.004 for β_{23}, β_{13}, and β_{12}, respectively. In Table 3.7, the correction approach WRC_E is shown to provide more efficiency by chance than the actual approach.

Table 3.7 Relative SEs of the estimators of the model parameters obtained using the actual, naive, RC_E, RC_A, WRC_E and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	1.013	2.985	2.989	2.987	1	1.004
β_2	1.012	2.102	2.103	2.104	1	1.004
β_3	0.999	1.437	1.443	1.444	1	0.990
β_{12}	1.007	1.735	1.755	1.755	0.994	0.994
β_{13}	1.013	1.590	1.596	1.596	1.003	1.003
β_{23}	1.009	1.542	1.549	1.549	1.004	1.003

Note that, even though the efficiency gain when using the WRC_E approach is high, the standard errors of the estimates of the model parameters are still higher than in the actual analysis, as shown in Table 3.5. In addition, from Table 3.6, the mean relative bias in the naive estimates may be so relatively small as to not matter in practice; however, the bias depends mainly on the size of the errors. This can be carefully considered before aiming to correct the bias in the naive estimates, as the mixing error variance could be quite small in some experimental situations.

To examine the prediction variance in each type of regression, seven selected design points are used. These are shown in Table 3.8.

Table 3.8 Number of selected design points used to examine the prediction variances.

	Proportions		
Run	w_1	w_2	w_3
1	0.40	0.40	0.20
2	0.45	0.30	0.15
3	0.35	0.35	0.30
4	0.40	0.30	0.30
5	0.30	0.40	0.30
6	0.30	0.30	0.30
7	0.50	0.20	0.30

The averaged prediction variances from fitting the naive, RC_E, RC_A, WRC_E, and WRC_A, over 10^4 simulations are 2.605, 2.412, 2.413, 1.301 and 1.301, respectively. Therefore, using the WRC_E and WRC_A reduce the prediction variance by approximately half compared to using the naive, RC_E, and RC_A approaches. Both of the approaches RC_E and RC_A also show a slight improvement in the prediction variance, but this can be considered insignificant, compared to that obtained from the WRC_E and WRC_A approaches.

3.5.4 Robustness of the Adjustment Approaches to the Misspecification of Mixture Error Variance

Most of the adjustment approaches used to compensate for the effect of EIV depend on the estimated value of the error variance. This estimate can be obtained prior to the adjustment analysis. In some situations, the error variance could be misspecified or inaccurately estimated. Hence, it is important to question the robustness of the adjusted approaches to misspecified errors.

The error variance could be underestimated or overestimated. Underestimating it means that the errors at the adjustment stage of the analysis are considered smaller than their actual values. Overestimating means that those errors are considered larger than their actual values.

The robustness of the RC approach to error misspecification has been investigated before in the literature. Mallick et al. (2002) studied the robustness of RC under the assumption of classical EIV. Their study showed that the robustness of the approach is highly jeopardized by error variance misspecification. For example, when the error variance is overestimated, the variances of the estimators obtained using the RC approach are also overestimated. Likewise, when the error variance is underestimated, the variances of the estimators in the RC are underestimated.

In the following we investigate the effectiveness of the adjusted approaches when there is error misspecification. For the misspecified values of the errors variances, a sensible range has been chosen, determined by practical considerations, and wider ranges would not be of interest but ones up to those limits are of interest.

For the approaches WRC_E and WRC_A, we assume that the response variance σ_ε^2 is known, and not misspecified.

3.5.4.1 Underestimating the Mixture Error Variance

Assume the components of a mixture are mixed with errors. Suppose the error variances are underestimated. A Monte Carlo simulation is conducted to examine the robustness of the corrected approaches RC_E, RC_A, WRC_E, and WRC_A for this error misspecification.

3.5.4.2 Simulation Example

Design Choice and Simulation Parameters: We use the simulation settings in the previous example with a slight change in the simulation parameters. Similar to the previous example, the required amounts of the three mixture components are discharged with errors, assumed to be normally distributed with zero means and variances σ_1^2, σ_2^2, and σ_3^2, $t = 1,2,\ldots,n$, respectively, where $\sigma_1 = 0.07$, $\sigma_2 = 0.08$, and $\sigma_3 = 0.10$. The variances are proportional to the squares of the discharged amounts, that is the variances of the actual amounts are $W_{1t}^2\sigma_1^2$, $W_{2t}^2\sigma_2^2$, and $W_{3t}^2\sigma_3^2$, respectively. However, to simulate the case of underestimating the errors, we assume that the standard deviations of the errors are underestimated as half of their actual size, that is $\hat{\sigma}_1 = \sigma_1/2$, $\hat{\sigma}_2 = \sigma_2/2$, and $\hat{\sigma}_3 = \sigma_3/2$. Thus, the values of the error standard deviations, to be used in the adjustment approaches, are $\hat{\sigma}_1 = 0.035$, $\hat{\sigma}_2 = 0.04$, and $\hat{\sigma}_3 = 0.05$.

Simulation Results: Tables 3.9, 3.10, 3.11, and 3.12, report the results of 10^4 Monte Carlo simulations of the underestimated error variance. The target is to compare the robustness of the adjustment approaches. The results illustrate the bias, the averaged model-based SE, the Monte Carlo SE, the percentage of absolute mean relative bias, and the relative standard error, respectively, of the estimators of β_1, β_2, β_3, β_{12}, β_{13}, and β_{23} obtained using the actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches.

Table 3.9 Investigating the robustness of the adjustment approaches to underestimated errors ($\hat{\sigma}_1 = 0.035$, $\hat{\sigma}_2 = 0.04$, and $\hat{\sigma}_3 = 0.05$). Bias in the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	-2.613×10^{-5}	-4.040×10^{-2}	-3.736×10^{-2}	-2.751×10^{-2}	-1.291×10^{-5}	-1.258×10^{-5}
β_2	-4.178×10^{-5}	-5.209×10^{-2}	-3.898×10^{-2}	-4.191×10^{-2}	-4.833×10^{-5}	-4.834×10^{-5}
β_3	-8.214×10^{-5}	0.133	0.111	-8.423×10^{-2}	-1.374×10^{-4}	-1.380×10^{-4}
β_{12}	9.214×10^{-5}	-1.619	-1.221	-1.205	-1.148	-1.136
β_{13}	5.782×10^{-4}	-0.998	-0.749	-0.761	-0.795	-0.810
β_{23}	1.470×10^{-4}	-1.113	-0.842	-0.830	-1.415	-1.415

The results show that the impact of misspecified error variance on the robustness of the adjustment approaches is generally high. The estimates of the binary components β_{23}, β_{13}, and β_{12} obtained using RC_E, RC_A, WRC_E, and WRC_A are the most affected. In particular, when the error variance is assumed known, the adjustment approaches WRC_E and WRC_A produce approximately unbiased and efficient estimates for the pure and binary component parameters. When the error variance is underestimated, the same approaches produce unbiased and efficient estimates only for the pure components. Estimates of the binary components, on the other hand, show some bias and reduction in efficiency. For example, the bias in β_{23} from WRC_E and WRC_A is -1.415, in the mean, while the bias in the naive estimate is -1.113. This can clearly be seen in Table 3.11 by comparing the percentages of the absolute mean relative biases in the estimates obtained from the adjustment approaches and the naive analysis. The approaches WRC_E and WRC_A underestimate the SEs of the estimators of the model parameters. However, their estimates still have a relatively higher efficiency than those from

the naive analysis. For example, in Table 3.12, the relative standard errors for β_{12} from the naive, WRC_E, and WRC_A approaches are 1.735, 0.467, and 0.468, respectively.

Table 3.10 Investigating the robustness of the adjustment approaches to underestimated errors ($\hat{\sigma}_1 = 0.035$, $\hat{\sigma}_2 = 0.04$, and $\hat{\sigma}_3 = 0.05$). The MC SE (the averaged model-based SE) of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	9.118×10^{-3} (9.234×10^{-3})	1.360 (4.060)	1.359 (4.059)	1.369 (4.057)	9.960×10^{-3} (9.959×10^{-3})	9.960×10^{-3} (1.000×10^{-2})
β_2	9.117×10^{-3} (9.231×10^{-3})	1.931 (4.060)	1.931 (4.057)	1.927 (4.058)	9.990×10^{-3} (9.989×10^{-3})	9.990×10^{-3} (1.003×10^{-2})
β_3	8.229×10^{-3} (8.217×10^{-3})	2.515 (3.615)	2.513 (3.615)	2.511 (3.616)	1.001×10^{-2} (1.001×10^{-2})	1.001×10^{-2} (9.915×10^{-3})
β_{12}	3.959×10^{-2} (3.989×10^{-2})	10.070 (17.470)	10.022 (17.479)	11.295 (17.479)	7.177 (3.354)	7.176 (3.356)
β_{13}	3.737×10^{-2} (3.787×10^{-2})	10.453 (16.616)	10.441 (16.626)	10.910 (16.626)	6.822 (3.308)	6.823 (3.310)
β_{23}	3.752×10^{-2} (3.786×10^{-2})	10.773 (16.616)	10.760 (16.626)	11.373 (16.624)	3.636 (1.194)	3.636 (1.193)

Table 3.11 Investigating the robustness of the adjustment approaches to underestimated errors ($\hat{\sigma}_1 = 0.035$, $\hat{\sigma}_2 = 0.04$, and $\hat{\sigma}_3 = 0.05$). Percentage of absolute mean relative bias of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	1.045×10^{-5}	1.616×10^{-2}	-1.494×10^{-2}	-1.100×10^{-2}	-5.163×10^{-6}	-5.033×10^{-6}
β_2	2.387×10^{-5}	2.977×10^{-2}	-2.227×10^{-2}	-2.395×10^{-2}	-2.762×10^{-5}	-2.763×10^{-5}
β_3	4.323×10^{-5}	7.000×10^{-2}	5.846×10^{-2}	4.433×10^{-2}	-7.232×10^{-5}	-7.263×10^{-5}
β_{12}	1.675×10^{-5}	0.294	-0.222	-0.219	-0.209	-0.206
β_{13}	1.522×10^{-4}	0.263	-0.197	-0.200	-0.209	-0.213
β_{23}	3.267×10^{-5}	0.247	-0.187	-0.185	-0.314	-0.314

Table 3.12 Investigating the robustness of the adjustment approaches to underestimated errors ($\hat{\sigma}_1 = 0.035$, $\hat{\sigma}_2 = 0.04$, and $\hat{\sigma}_3 = 0.05$). Relative standard error of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	1.013	2.985	2.986	2.965	1	1.004
β_2	1.012	2.102	2.102	2.106	1	1.004
β_3	0.999	1.437	1.438	1.440	1	0.990
β_{12}	1.007	1.735	1.744	1.547	0.467	0.468
β_{13}	1.013	1.590	1.592	1.524	0.485	0.485
β_{23}	1.009	1.542	1.545	1.462	0.328	0.328

3.5.4.3 Overestimating the Mixture Error Variance

Assume the components of a mixture are mixed with errors. Suppose the error variances are overestimated. A Monte Carlo simulation is conducted to examine the robustness of the corrected approaches RC_E, RC_A, WRC_E, and WRC_A to this error misspecification.

3.5.4.4 Simulation Example

Design Choice and Simulation Parameters: Similar to the previous simulation study, we only change the error standard deviation in the simulated experiment. The required amounts of the three mixture components are discharged with errors, assumed to be normally distributed with zero means and variances σ_1^2, σ_2^2, and σ_3^2, $t = 1,2,\ldots,n$, respectively, where $\sigma_1 = 0.07$, $\sigma_2 = 0.08$, and $\sigma_3 = 0.10$. The variances are proportional to the squares of the discharged amounts, that is the variances of the actual amounts are $W_{1t}^2\sigma_1^2$, $W_{2t}^2\sigma_2^2$, and $W_{3t}^2\sigma_3^2$, respectively. However, to simulate the case of overestimating the error size, we assume the error standard deviations to be overestimated as twice their actual size, that is $\hat{\sigma}_1 = 2\sigma_1$, $\hat{\sigma}_2 = 2\sigma_2$, and $\hat{\sigma}_3 = 2\sigma_3$. Thus the values of the error standard deviations to be used in the adjustment approaches are $\hat{\sigma}_1 = 0.14$, $\hat{\sigma}_2 = 0.16$, and $\hat{\sigma}_3 = 0.20$.

Simulation Results: Tables 3.13, 3.14, 3.15, and 3.16 report the results of running 10^4 Monte Carlo simulations for the overestimated error variances. The results illustrate the bias, average model-based SE, Monte Carlo SE, percentage of absolute relative bias, and relative standard error, respectively, of the estimators of β_1, β_2, β_3, β_{12}, β_{13}, and β_{23} obtained using the actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches.

Table 3.13 Investigating the robustness of the adjustment approaches to overestimated errors ($\hat{\sigma}_1 = 0.14$, $\hat{\sigma}_2 = 0.16$, and $\hat{\sigma}_3 = 0.20$). Bias of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	-2.613×10^{-5}	-4.040×10^{-2}	0.154	0.176	-1.143×10^{-5}	-1.140×10^{-5}
β_2	-4.178×10^{-5}	-5.209×10^{-2}	0.139	0.122	-4.592×10^{-5}	-4.594×10^{-5}
β_3	-8.214×10^{-5}	0.133	-0.542	-0.641	-1.397×10^{-4}	-1.398×10^{-4}
β_{12}	9.214×10^{-5}	-1.619	5.199	5.038	4.913	4.754
β_{13}	5.782×10^{-4}	-0.998	2.949	2.750	3.442	3.178
β_{23}	1.470×10^{-4}	-1.113	3.663	3.372	5.099	4.667

Table 3.14 Investigating the robustness of the adjustment approaches to overestimated errors ($\hat{\sigma}_1 = 0.14$, $\hat{\sigma}_2 = 0.16$, and $\hat{\sigma}_3 = 0.20$). The MC SE (the average model-based SE) of the estimators of the model parameters obtained using the actual, naive, RC_E , RC_A , WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	9.118×10^{-3}	1.360	1.364	1.369	9.959×10^{-3}	9.959×10^{-3}
	(9.234×10^{-3})	(4.060)	(4.093)	(4.099)	(9.959×10^{-3})	(1.000×10^{-2})
β_2	9.117×10^{-3}	1.931	1.929	1.927	9.989×10^{-3}	9.989×10^{-3}
	(9.231×10^{-3})	(4.060)	(4.089)	(4.096)	(9.989×10^{-3})	(1.003×10^{-2})
β_3	8.229×10^{-3}	2.515	2.548	2.568	1.001×10^{-2}	1.001×10^{-2}
	(8.217×10^{-3})	(3.615)	(3.663)	(3.671)	(1.001×10^{-2})	(9.915×10^{-2})
β_{12}	3.959×10^{-2}	10.070	11.369	11.295	8.717	8.628
	(3.989×10^{-2})	(17.47)	(17.813)	(17.836)	(17.103)	(17.113)
β_{13}	3.737×10^{-2}	10.453	10.965	10.910	7.748	7.631
	(3.787×10^{-2})	(16.62)	(16.972)	(16.986)	(15.378)	(15.377)
β_{23}	3.752×10^{-2}	10.773	11.478	11.373	6.348	6.004
	(3.786×10^{-2})	(16.62)	(16.947)	(16.954)	(12.200)	(12.173)

The results show that the impact of the overestimated errors on the analysis is larger than that in the case of the underestimated errors. The estimates of the binary components from the same approaches are highly affected when the errors are overestimated. The biases in the estimators of β_{12}, β_{13}, and β_{23} obtained using RC_E, RC_A, WRC_E, and WRC_A are much larger than the biases in the naive estimates. For example, the biases in the estimates of β_{12} obtained using the naive and WRC_E approaches are -1.619 and 4.913, respectively. The results in Table 3.15 support these figures, with increased percentages of absolute relative biases in the estimates of the binary components. From Table 3.16 it can be seen that the relative standard errors for the main effects obtained using the naive, WRC_E, and WRC_A approaches are 2.985,

1, and 1.004, respectively, which shows an improvement in the efficiency of the estimators. Hence, similar to the case of the underestimated errors, the adjusted estimates for the pure components obtained using WRC_E and WRC_A are unbiased and efficient compared to the naive ones.

In addition, from Tables 3.14 and 3.16, we can see that the standard errors of the adjusted estimators are overestimated. For the binary effects, for example, the relative SEs of the estimators of the binary effect β_{12} obtained using the naive, WRC_E, and WRC_A approaches are 1.735, 1.962, and 1.983, respectively. The same applies to the binary components β_{23} and β_{13}.

Table 3.15 Investigating the robustness of the adjustment approaches to overestimated errors ($\hat{\sigma}_1 = 0.14$, $\hat{\sigma}_2 = 0.16$, and $\hat{\sigma}_3 = 0.20$). Percentage of absolute mean relative bias of the estimators of the model parameters obtained using the actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	1.045×10^{-5}	1.616×10^{-2}	6.156×10^{-2}	0.070	-4.572×10^{-6}	-4.560×10^{-6}
β_2	2.387×10^{-5}	2.977×10^{-2}	0.079	0.0699	-2.624×10^{-5}	-2.625×10^{-5}
β_3	4.323×10^{-5}	7.000×10^{-2}	-0.285	-0.337	-7.354×10^{-5}	-7.361×10^{-5}
β_{12}	1.675×10^{-5}	0.294	0.945	0.916	0.893	0.864
β_{13}	1.522×10^{-4}	0.263	0.776	0.724	0.906	0.836
β_{23}	3.267×10^{-5}	0.247	0.814	0.749	1.133	1.037

Table 3.16 Investigating the robustness of the adjustment approaches to overestimated errors ($\hat{\sigma}_1 = 0.14$, $\hat{\sigma}_2 = 0.16$, and $\hat{\sigma}_3 = 0.20$). Relative standard error of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	1.013	2.985	3.001	2.994	1	1.004
β_2	1.012	2.102	2.120	2.125	1	1.004
β_3	0.999	1.437	1.438	1.430	1	0.990
β_{12}	1.007	1.735	1.567	1.579	1.962	1.983
β_{13}	1.013	1.590	1.548	1.557	1.985	2.015
β_{23}	1.009	1.542	1.476	1.491	1.922	2.028

3.5.5 Robustness of WRC_E and WRC_A Approaches to a Misspecification of the Response Error Variance

The approaches WRC_E and WRC_A rely on the assumption of a known response error variance σ_ε^2. However, in practice, this assumption may not always be valid and an estimate of σ_ε^2 will have to be obtained in order to use these approaches. In this section, an investigation into the effect of underestimating or overestimating σ_ε^2 is presented. The effect of misspecifying the variance is studied by comparing the naive, RC_E, and RC_A approaches to the corrected approaches, WRC_E and WRC_A. In the previous simulations, the response errors were assumed to be small (that is σ_ε=0.01) so that more focus could be given to the effect of the mixing errors on the analysis. Since now the attention is given to the effect of any response error misspecification, assuming σ_ε=0.01 would be misleading as the errors would be too small to reveal any problems if the variance was unknown. Thus, a larger value of σ_ε^2 is used in the following simulation examples. For the misspecified values of the error variances, a sensible range has been chosen, determined by practical considerations, and wider ranges would not be of interest but ones up to those limits are of interest.

3.5.5.1 Underestimating the Response Error Variance

Assume the components of a mixture are measured with errors. Suppose the error variances are well estimated but the response errors are underestimated. A Monte Carlo simulation is conducted to examine the robustness of the corrected approaches WRC_E and WRC_A to such an error misspecification.

3.5.5.2 Simulation Example

Design Choice and Simulation Parameters: Assume the same simulation setting as in Section 3.5.3. Let σ_ε =5.48. To simulate the case where we underestimate the response errors, we assume that the standard deviation of the errors is underestimated as half of its actual size, that is $\hat{\sigma}_\varepsilon = \sigma_\varepsilon / 2$.

Simulation Results: Tables 3.17, 3.18, 3.19, and 3.20 report the results of running 10^4 Monte Carlo simulations of underestimated response error variance. The results illustrate the bias, the average model-based and Monte Carlo standard errors, the percentage of absolute relative bias, and the relative standard errors, respectively, of the estimators of β_1, β_2, β_3, β_{12}, β_{13}, and β_{23} obtained using the actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches.

In general, Tables 3.17 and 3.19 show that, particularly when estimating the parameters β_{12}, β_{13}, and β_{23}, the corrected approaches give unbiased estimators compared to the estimators obtained using the naive approach and their values are approximately as good as those produced from the actual analysis of the data. Thus, even though the approaches WRC_E and WRC_A have been used with misspecified σ_ε, their estimators are still approximately unbiased in comparison to the true parameters. However, from Tables 3.18 and 3.20, it can be seen that the performances of WRC_E and WRC_A are not as efficient as the naive approach. For example, in Table 3.20, for $\beta_{12,}$ the relative SEs obtained using the naive and WRC_A approaches are 0.571 and 1.175, respectively. In addition, unlike in the case where σ_ε is known, no reduction in the true variance of the estimators is obtained.

Table 3.17 Investigating the robustness of the adjustment approaches to underestimated response error variance ($\hat{\sigma}_\varepsilon = 2.74$). Bias in the estimators of the model parameters obtained using actual, naive analysis and RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	-1.432×10^{-2}	-5.315×10^{-2}	-2.001×10^{-2}	-1.098×10^{-3}	-4.741×10^{-3}	1.881×10^{-3}
β_2	-2.289×10^{-2}	-7.615×10^{-2}	-2.878×10^{-2}	-3.486×10^{-2}	-2.367×10^{-2}	-2.448×10^{-2}
β_3	-4.501×10^{-2}	9.107×10^{-2}	-5.025×10^{-2}	-0.104	-6.564×10^{-2}	-8.051×10^{-2}
β_{12}	5.049×10^{-2}	-1.550	8.817×10^{-2}	0.107	1.462×10^{-2}	3.047×10^{-2}
β_{13}	0.317	-0.711	0.260	0.234	0.291	0.263
β_{23}	8.055×10^{-2}	-1.038	8.804×10^{-2}	9.229×10^{-2}	5.242×10^{-2}	2.495×10^{-2}

Table 3.18 Investigating the robustness of the adjustment approaches to underestimated response error variance ($\hat{\sigma}_\varepsilon = 2.74$). The MC SE (the average model-based SE) of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A , WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	4.997 (5.060)	5.194 (6.490)	5.195 (6.491)	5.195 (6.491)	5.204 (2.641)	5.204 (2.652)
β_2	4.996 (5.058)	5.380 (6.490)	5.379 (6.489)	5.379 (6.490)	5.307 (2.685)	5.305 (2.695)
β_3	4.510 (4.503)	5.148 (5.780)	5.151 (5.786)	5.152 (5.789)	5.067 (2.605)	5.075 (2.584)
β_{12}	21.697 (21.858)	23.781 (27.931)	23.806 (28.008)	23.809 (28.012)	23.816 (13.534)	23.812 (13.599)
β_{13}	20.481 (20.752)	22.916 (26.566)	22.984 (26.650)	22.985 (26.650)	22.848 (12.975)	22.826 (13.065)
β_{23}	20.559 (20.746)	23.137 (26.566)	23.189 (26.642)	23.185 (26.638)	22.935 (12.271)	22.853 (12.418)

Table 3.19 Investigating the robustness of the adjustment approaches to underestimated response error variance ($\hat{\sigma}_\varepsilon = 2.74$). Percentage of absolute mean relative bias of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	5.727×10^{-3}	2.126×10^{-2}	8.006×10^{-3}	4.392×10^{-4}	1.896×10^{-3}	7.524×10^{-4}
β_2	1.308×10^{-2}	4.352×10^{-2}	1.645×10^{-2}	1.992×10^{-2}	1.353×10^{-2}	1.399×10^{-2}
β_3	2.369×10^{-2}	4.793×10^{-2}	2.645×10^{-2}	5.492×10^{-2}	3.455×10^{-2}	4.238×10^{-2}
β_{12}	9.180×10^{-3}	0.282	1.603×10^{-2}	1.940×10^{-2}	2.659×10^{-3}	5.540×10^{-3}
β_{13}	8.339×10^{-2}	0.187	6.832×10^{-2}	6.162×10^{-2}	7.653×10^{-2}	6.917×10^{-2}
β_{23}	1.790×10^{-2}	0.231	1.956×10^{-2}	2.051×10^{-2}	1.165×10^{-2}	5.544×10^{-3}

Table 3.20 Investigating the robustness of the adjustment approaches to underestimated response error variance ($\hat{\sigma}_\varepsilon = 2.74$). Relative standard errors of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	1.013	1.250	1.249	1.249	0.507	0.510
β_2	1.012	1.206	1.206	1.206	0.506	0.508
β_3	0.999	1.123	1.123	1.123	0.514	0.509
β_{12}	1.007	1.175	1.177	1.176	0.568	0.571
β_{13}	1.013	1.159	1.159	1.159	0.568	0.572
β_{23}	1.009	1.148	1.149	1.149	0.535	0.543

3.5.5.3 Overestimating the Response Error Variance

Assume that the components of a mixture are measured with errors. Suppose the error variances are well estimated but the response errors are overestimated. A Monte Carlo simulation is conducted to examine the robustness of the corrected approaches WRC_E and WRC_A to such an error misspecification.

3.5.5.4 Simulation Example

Design Choice and Simulation Parameters: Assume the same simulation setting as in Section 3.5.3. Let σ_ε=5.48. To simulate the case of overestimating the response errors, we assume that the standard deviation of the errors is estimated to be twice its actual size, that is, $\hat{\sigma}_\varepsilon = 2\sigma_\varepsilon$.

Simulation Results: Tables 3.21, 3.22, 3.23, and 3.24 report the results of running 10^4 Monte Carlo simulations of the overestimated response error variance. The results illustrate the bias, average model-based and Monte Carlo standard errors, the percentage of absolute relative bias, and the relative standard errors, respectively, of the estimators of β_1, β_2, β_3, β_{12}, β_{13}, and β_{23} obtained using the actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches.

Table 3.21 Investigating the robustness of the adjusted approaches to overestimated response error variance ($\hat{\sigma}_\varepsilon = 10.96$). Biases in the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	-1.432×10^{-2}	-5.315×10^{-2}	-2.001×10^{-2}	-1.098×10^{-3}	-1.542×10^{-2}	1.419×10^{-3}
β_2	-2.289×10^{-2}	-7.615×10^{-2}	-2.878×10^{-2}	-3.486×10^{-2}	-2.551×10^{-2}	-3.038×10^{-2}
β_3	-4.501×10^{-2}	9.107×10^{-2}	-5.025×10^{-2}	-0.104	-5.075×10^{-2}	-9.623×10^{-2}
β_{12}	5.049×10^{-2}	-1.550	8.817×10^{-2}	0.107	6.589×10^{-2}	8.418×10^{-2}
β_{13}	0.317	-0.711	0.260	0.234	0.252	0.224
β_{23}	8.055×10^{-2}	-1.038	8.804×10^{-2}	9.229×10^{-2}	7.855×10^{-2}	7.368×10^{-2}

Again, the RC_E, RC_A, WRC_E, and WRC_A approaches all showed improvements over the naive approach in terms of a correction of the bias in the estimators of the model parameters. However, their estimators were found to be inefficient. In particular, the WRC_E and WRC_A approaches that depend on the value of the response error variance were highly affected by the misspecification of σ_ε^2. Their estimators were even more inefficient than the naive estimates of the model parameters. Therefore, it is preferable to use the WRC approaches only if σ_ε^2 is known or can be accurately estimated.

Table 3.22 Investigating the robustness of the adjusted approaches to overestimated response error variance ($\hat{\sigma}_\varepsilon = 10.96$). The MC SE (the average model-based SE) of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	4.997 (5.060)	5.194 (6.490)	5.195 (6.491)	5.195 (6.491)	5.162 (10.143)	5.163 (10.181)
β_2	4.996 (5.058)	5.380 (6.490)	5.379 (6.489)	5.379 (6.490)	5.281 (10.241)	5.288 (10.270)
β_3	4.510 (4.503)	5.148 (5.780)	5.151 (5.786)	5.152 (5.789)	5.004 (9.301)	4.990 (9.256)
β_{12}	21.697 (21.858)	23.781 (27.931)	23.806 (28.008)	23.809 (28.012)	23.596 (44.581)	23.615 (44.600)
β_{13}	20.481 (20.752)	22.916 (26.566)	22.984 (26.650)	22.985 (26.650)	22.612 (42.539)	22.623 (42.664)
β_{23}	20.559 (20.746)	23.137 (26.566)	23.189 (26.642)	23.185 (26.638)	22.624 (42.494)	22.678 (42.737)

Table 3.23 Investigating the robustness of the adjusted approaches to overestimated response error variance ($\hat{\sigma}_\varepsilon = 10.96$). Percentage of absolute mean relative bias of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	5.727×10^{-3}	2.126×10^{-2}	8.006×10^{-3}	4.392×10^{-4}	6.169×10^{-3}	5.675×10^{-4}
β_2	1.308×10^{-2}	4.352×10^{-2}	1.645×10^{-2}	1.992×10^{-2}	1.458×10^{-2}	1.736×10^{-2}
β_3	2.369×10^{-2}	4.793×10^{-2}	2.645×10^{-2}	5.492×10^{-2}	2.671×10^{-2}	5.065×10^{-2}
β_{12}	9.180×10^{-3}	0.282	1.603×10^{-2}	1.940×10^{-2}	1.198×10^{-2}	1.531×10^{-2}
β_{13}	8.339×10^{-2}	0.187	6.832×10^{-2}	6.162×10^{-2}	6.629×10^{-2}	5.905×10^{-2}
β_{23}	1.790×10^{-2}	0.231	1.956×10^{-2}	2.051×10^{-2}	1.746×10^{-2}	1.637×10^{-2}

Table 3.24 Investigating the robustness of the adjusted approaches to overestimated response error variance ($\hat{\sigma}_\varepsilon = 10.96$). Relative standard error of the estimators of the model parameters obtained using actual, naive, RC_E, RC_A, WRC_E, and WRC_A approaches, evaluated over 10^4 MC simulations.

	Actual	Naive	RC_E	RC_A	WRC_E	WRC_A
β_1	1.013	1.250	1.249	1.249	1.965	1.972
β_2	1.012	1.206	1.206	1.206	1.939	1.942
β_3	0.999	1.123	1.123	1.123	1.858	1.855
β_{12}	1.007	1.175	1.177	1.176	1.889	1.889
β_{13}	1.013	1.159	1.159	1.159	1.881	1.886
β_{23}	1.009	1.148	1.149	1.149	1.878	1.884

3.5.6 Characterizing a Mixture Product

Suppose that a consumer product (e.g. an alloy or fertilizer) that is to be manufactured is the mixture $\mathbf{w}_a$, and the manufacturing equipment is to be set up in this way. If mixing errors are unavoidable, Lemma 3.1 shows that $E[\mathbf{x}_a] \neq \mathbf{w}_a$, and therefore the average value of the response y, characterizing the product, will differ from what is intended, i.e. $E[y(\mathbf{x}_a)] \neq E[y(\mathbf{w}_a)]$. Hence, the average proportions of the components of the mixture will be different from the intended ones, the average characteristics of the product, represented by the response, will be biased, and the variability of y will be increased. Clearly this could have serious implications for the overall quality of the product. Similar effects could be observed if

the operating conditions under which the product is mixed cause errors in the proportions of the mixture components, due to dilution or degradation. For example, this phenomenon can occur in bioassay screening.

The results of Section 3.3.1 can help us to find a solution to this problem. For example, the simple model (3.3) will usually be sufficient to predict the response in the neighborhood of the mixture $\mathbf{w}_a$ and can be obtained experimentally given the choice of $\mathbf{w}_a$. From Theorem 3.1 it follows that $E[y \mid \mathbf{w}_a] = \eta(\boldsymbol{\beta}, \mathbf{w}_a) + B$. It is therefore possible to set up the equipment to manufacture a mixture, say $\mathbf{w}_m$ (rather than $\mathbf{w}_a$) such that $E[y \mid \mathbf{w}_m] \approx \eta(\boldsymbol{\beta}, \mathbf{w}_a)$. From Theorem 3.1 it follows that the mixture $\mathbf{w}_m$ should satisfy the identity

$$\sum_{i=1}^{q} \beta_i \left(w_{im} - w_{ia} \right) + T^{-2} \sum_{i=1}^{q} \beta_i \left[(w_{im} - 1) \varphi_i(W_{im}) \sigma_i^2 + w_{im} \sum_{j \neq i}^{q} \varphi_j(W_{jm}) \sigma_j^2 \right] = 0 . \qquad (3.15)$$

In general, an infinite number of mixtures satisfy this identity, and hence the one chosen for the manufacturing process could satisfy other considerations. For example, the variability of the responses associated with different mixtures will differ, and therefore the mixture for which this variability is the smallest may be the most attractive choice. Equation (3.10) which follows from Theorem 3.2 can be used to make the choice.

3.5.7 Simulation Example

Design Choice and Simulation Parameters: Assume that the desirable values of a response, *y*, of a three-component mixture would be obtained if the manufactured mixture was $\mathbf{w}_a = (0.5,\ 0.2,\ 0.3)$. However, we expect there to be mixing errors with mean zero and variances σ_1^2, σ_2^2, and σ_3^2, that is the variances of the actual amounts are $W_{1m}^2 \sigma_1^2$, $W_{2m}^2 \sigma_2^2$, and $W_{3m}^2 \sigma_3^2$, respectively, where $\sigma_1 = 0.07$, $\sigma_2 = 0.08$, and $\sigma_3 = 0.10$. Suppose also that model (3.3) can be used to describe the response in the neighborhood of $\mathbf{w}_a$, with $\boldsymbol{\beta}^{\mathbf{T}} = (250,\ 175,\ 190)$. Large numbers of design points are generated in the area of the target design point $\mathbf{w}_a$

using a step of 1×10^{-5}. This step will be narrowed by examining each of the points in order to find the one that satisfies equation (3.15). Only the points that give a root of less than 5×10^{-6} for equation (3.15) are selected, since in our example, we cannot find a point in the neighborhood of $\mathbf{w}_a$ that gives us an exact root of zero for equation (3.15). However, we did find that in other simulated experiments this condition was satisfied when small errors and model parameters were assumed.

Simulation Results: Calculations using equation (3.15) show that there are many other mixtures that result in products with the average desired response. However, we pick only three of the mixtures that satisfy equation (3.15). These points are: (0.501, 0.201, 0.298), (0.501, 0.202, 0.297), and (0.501, 0.205, 0.294). The response variances resulting from using these three points are the smallest out of all the generated design points and are given approximately by 2.4451, 2.4454, and 2.4467 respectively. However, the mixtures (0.501, 0.201, 0.298) and (0.501, 0.202, 0.297) ensure that the variability of the response is minimized (since their response variances seem to be close in value). This mixture should therefore be recommended to the manufacturer.

3.6 Summary and Discussion

In this chapter, analytical and empirical results were developed to address the effects of mixing errors on the analysis of mixture experiments. The results show that ignoring mixing errors leads to biased and inefficient ordinary least squares estimators of the model parameters. The direction and size of the bias depend on many factors, but mainly on the size of the mixing error variances.

As a result of the mixing errors, the model thus becomes biased with increased and heterogeneous variance. A method based on the RC and WLS approaches was proposed. We also implemented a method based on the RC approach alone. The form of the bias is very

important in the application of the RC approach. If the variances of the mixing errors and σ_ε^2 can be obtained prior to the experiment, these values can be used to develop a calibrated extended design matrix $\mathbf{F}_c$, as well as estimates for the variances of the responses at the design points. Then, the estimator of the model parameters $\hat{\boldsymbol{\beta}}_{WRC}$, provides better estimates of the model parameters than those obtained by ignoring the mixing errors. Generally, finding $\mathbf{F}_c$ analytically is difficult. In our examples, we have shown that a calibrated extended design matrix $\mathbf{F}_c$, as well as standard errors for the corrected model parameters can be obtained by computer simulation. The correction approach WRC that we propose produces approximately unbiased and efficient estimators.

The results we obtain are novel in regards to mixture experiments. However, they might be comparable with some of the previous studies in the area, particularly multiple linear regression models with EIV. In mixture experiments, if second or higher order Scheffé polynomials are used to fit the data from a mixture experiment, and mixing errors occurs in at least one component, all the estimates of the coefficients will be biased. The direction of the bias is unpredictable. A similar effect occurs in multiple linear regression models with errors in at least one of the variables, for example see Buzas et al. (2004).

When the error variance is misspecified, the robustness of the approaches used to estimate the binary effects is reduced. Particularly for overestimated error variance, the estimates of the model parameters obtained using the adjustment approaches have enlarged biases and overestimated variances. Most of the estimates of model parameters obtained using underestimated error variance encountered biases and underestimated variance, but the biases were not larger than the biases in the naive estimates for most of the binary effects. Note that, the adjustment approaches WRC_E and WRC_A can be considered robust for estimating the pure components β_1, β_2, and β_3 even with misspecified error variance. Their estimates are unbiased and efficient. Thus, if it is suspected that the error variance has been misspecified in the

experiment, we recommend using the WRC_E and WRC_A approaches only, to fit the pure components in any response model. For example, if the model to be fitted is a first-order polynomial, then the estimates of the model parameters will be unbiased and efficient even with misspecified error variance.

A disadvantage of our correction approaches is that they are based on approximations of the expectations of the true values, given the observed values. To find the appropriate approximations, strong distributional assumptions need to be imposed. Hence any misspecification of such assumptions could lead to invalid approximations. However, we have to point out that RC, which is one of the most commonly used correction methods, also relies on such approximations and faces the same problem as our approaches when the errors are misspecified.

When applying the proposed approaches, a possible reason for inconsistency in the approximations is that the number of samples generated to obtain the corrected extended design matrix might not have been sufficiently large. Thus, the use of a large number of replications is always recommended. However, the number should only be increased if the additional computational effort is reasonable.

Some of the methods proposed only reduced the bias in the naive estimates and some others reduced the bias and produced efficient estimates. The choice of appropriate method should be based on what assumptions can be made regarding the error components in the EIV model.

The bias in the naive estimates of the model parameters was found to be relatively small. Despite the fact that in the literature on EIV, some researchers tend to ignore small biases in the estimates of parameters, we believe that there are special cases in which it is essential to address this and produce accurate results, by correcting for biases of any magnitude. The biases in the estimates depend on the variance of the EIV so, with large variance, greater bias

will be found in the estimates. In practice, it is true that the variance is more likely to be small. However, large variances are possible if an experiment is not done very carefully. In addition, simulations show that, on average, we obtain the most consistent and efficient estimates of the model parameters by correcting the bias in the naive estimates. The bias is also a function of the model parameters so, with large parameter sizes, the effect of the error is found to be more significant.

Nevertheless, even though the effects of the mixing errors have been addressed, the results that we present show that their impact cannot be removed completely from the statistical analysis of the data. The increase in the variability of the results propagates to the accuracy of the estimation of the model parameters and the prediction of the response. This is a particularly important limitation when the aim of the experiment is to determine the manufacturing settings of a mixture product, and when mixing errors cannot be avoided. Selecting a mixture $\mathbf{w}_m$ for manufacturing, as described earlier, so that the manufacturing product has the asymptotic properties of the desired mixture $\mathbf{w}_a$, would eliminate the asymptotic bias. However, the variance of the response would remain increased. Therefore, a typical quality that was characteristic of the product, based on the signal to noise ratio, and therefore the quality of the product itself, would be reduced by the mixing errors. Hence, perhaps not surprisingly, trying to avoid or at least minimize mixing errors should be the first thing considered.

4 Berkson Errors in Bioassays

4.1 Introduction

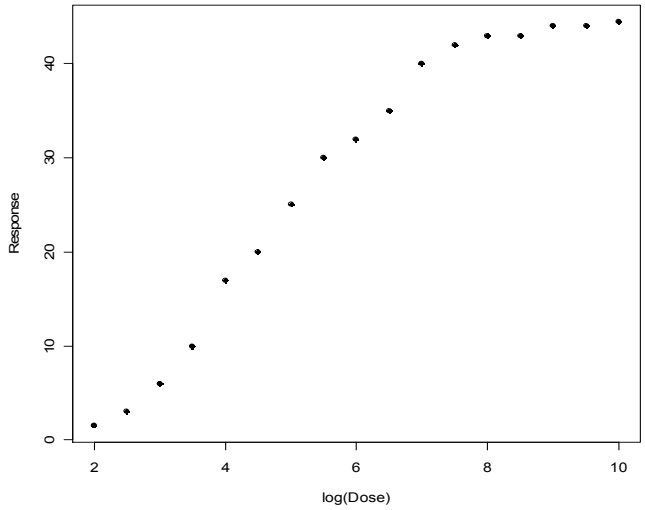
A bioassay can be defined as the screening, testing, and ranking of compounds. It has applications in drug discovery, medical research, and biological trials. Particularly, bioassays in drug discovery are aimed at specifying the minimum dose or concentration of a compound that shows a significant efficacy, while having negligible side effects. The studied effect is represented in what is called a response, and by analysing the relation between the response and dose, the effectiveness of the drug can be determined. In practice, commonly doses on the logarithmic scale are used when fitting the responses. An example of the typical effect of the increase of dose on the response is given in Figure 4.1.

In a drug discovery process (DDP), new drugs are compared with standard treatments. This process is usually carried out by large pharmaceutical companies. It starts by performing experimental pre-clinical (in vitro) and clinical (in vivo) trials. These trials are part of the DDP, which is a very expensive process running over a long period of time, e.g. 10–15 years.

Initially, large numbers of compounds are tested. Those without sufficient potencies are discarded. Consequently, smaller number of compounds proceed to additional tests. Only compounds that successfully complete the different phases of DDP can become drugs and be

sold on the market. These are compounds that have been shown to have high potency and negligible undesirable side effects (e.g. toxicity).

Figure 4.1 A typical effect of the increase of dose on the response.



As mentioned the DDP passes by two main phases: in vitro and in vivo. To illustrate the two phases, examples 1 and 2 are given.

4.1.1 Examples

Example 1. In Vitro Bioassay; A useful step in the DDP is the screening of compounds for cellular activities; see for example Molony (2002). The screening process is performed on the compound libraries by setting plates of chosen size (e.g. 96 wells plate), so that different concentrations of the studied compounds are tested in each well using the same experimental design for each compound. Often robots are used to pipette compounds into the wells and to record the responses, on the other hand this process could be done manually depending on the size of the assay study.

In these cases, an initial solution is prepared, often using a standard solvent and pipetted as required by the experimental design. The data could be then used to estimate the potency of each compound by fitting the appropriate response model. At the end of the in vitro study,

compounds are ranked depending on their potency, and those with the highest potency will be selected for further studies.

Example 2. In Vivo Bioassay; If the screening described in Example 1 has been successful, a small number of compounds that have shown desirable properties could be tested on animals. Compounds that have been showing promise can be also tested on a small number of healthy volunteers in order to examine safety aspects of the drug. Once these aspects are confirmed, larger number of individuals are selected to search for optimal dosing and scheduling of the new drugs. The new drug is also compared with other drugs on the market. Eventually the drug is applied to a large sample of patients with the target doses to monitor all aspects of the drug effects. For additional information about the DDP, see the summary in Triggle (2007).

4.1.2 Experimental Designs for Bioassays: Optimal Designs, Serial Dilution Designs

Bioassay studies are usually performed within a well-designed experiment. Experimental designs in bioassays are an approach to choose the number and values of target doses (or concentrations) of a compound for which the response is measured. Common designs for bioassays consist of observations of the response of interest for a number of equally distant on the logarithmic scale concentrations.

In practice, there are different ways to design a bioassay experiment. For example, the suitable design for a bioassay study can be selected either according to certain scientific criteria or using one's experience. Two main concepts in experiment design in bioassays are introduced briefly here. These concepts are optimal designs and serial dilution designs.

Optimal Designs: If the doses used in a bioassay experiment are chosen in such a way that particular statistical properties of the results are ensured, such a design is called an

optimum design. There are many criteria of optimality, such as, D- and G- optimality. For example, D-optimum designs are based on maximising the determinant of the information matrix for the design, so that the generalised variance of the estimates of the model parameters is minimised (Atkinson et al., 2007, p. 135). As a result of using D-optimal designs, inferences of parameters are found to be more efficient than if standard designs are used.

When the model used to fit the data from a bioassay is nonlinear, the determinant of the information matrix can only be calculated if the values of the model parameters are known (or approximately known). If these values are used to construct the D-optimum design, the design is then is said to be locally D-optimum. Such values might be available before the start of the experiment from experience or previous research.

Commonly in bioassay studies the same design is used to investigate a large number of compounds. Choosing the same design to test different compounds, will lead to no bias in estimates of potency, but the variance of the estimates will be inefficient. These designs have been used in many practical situations, even though the accuracy of the estimate of α is compromised. In such a case, the population D-optimality criteria defined by Donev and Tobias (2011) can be used to construct a suitable design.

Serial Dilution Designs: In vitro bioassays, designs often use serial pipetting of different concentrations of compounds into the wells of plates. These designs are called serial dilution designs (SDDs). The dilution process starts with pipetting an initial stock solution in a well (or wells if there are replications), to produce what is called the first or top concentration w_1. The next dose is obtained by reducing the previous by a dilution factor k. The dilution process will continue until the required number of doses is achieved. The choice of the suitable value for k usually depends on the case under study. Table 4.1 shows a SDD with 8 design doses, wherein w_1=32 and k=2.

Table 4.1 SDD with 8 design points, w_1=32 and k=2.

Design point	Dose
1	32
2	16
3	8
4	4
5	2
6	1
7	0.5
8	0.25

In bioassays, the true concentrations are seldomly known, and only the target concentrations are used in the statistical analysis of the data. This is therefore a typical scenario of a Berkson error problem. The errors are often proportional and related to the actual amounts; hence the assumption of heteroscedastic (non-constant) Berkson errors that can be seen as more sensible than homoscedastic errors.

When correcting the effects of the errors, most of the correction methods require some knowledge about the distribution of the EIV. The assumption of Gaussian, or normally distributed errors has been used many times. An exception is Wang et al. (2010), who studied the case of non-Gaussian EIV when a simple linear model has to be estimated, Wang (2006) in logistic regression and Suh and Schafer (2002) in nonlinear response model.

Bias correction in the case of Berkson Gaussian EIV when linear or generalised linear models have to be estimated has been studied by Burr (1988), Rudemo et al. (1989), Ridout and Fenlon (1991), Buonaccorsi and Lin (2002), Kim et al. (2006), Küchenhoff et al. (2007) and Althubaiti and Donev (2010).

The effect of EIV in bioassay has also been studied; see for example Racine-Poon et al. (1991), Dellaportas and Stephens (1995), Higgins et al. (1998), and Gelman et al. (2004). Again, EIV with normal distribution is assumed. It was reported that the naive analysis seriously underestimates the variability of the estimates of the parameters of the model, and that the response variance is inflated. Racine-Poon et al. (1991) recognised that EIV are often

proportional to the concentrations and developed a model to describe how the data is generated when a serial dilution design (SDD) is used and dilution errors in concentrations are made. The model is given as follows. Let w_t be the target concentrations by design, where $t = 1, 2, \ldots, n$. The dilution process starts by diluting a stock solution, say w_0 in m steps to obtain w_1 in a SDD. If the dilution factor k=2, the actual top concentration x_1 can be given by;

$$x_1 = 2^{-m} w_0 (1 + u_{w_0 1})(1 + u_{w_0 2}) \ldots (1 + u_{w_0 m}), \tag{4.4}$$

where the dilution errors $u_{w_0 1}, u_{w_0 2}, \ldots, u_{w_0 m}$ associated with diluting the initial solution w_0 are assumed to be identically and independently normally distributed with mean zero and small variance σ_u^2. If we fit the model in the logarithmic scale to base 10 of the concentration of the compound, equation (4.4) gives $\log x_1 = \log w_1 + \sum_{j=1}^{m} \log(1 + u_{w_0 j})$. If the errors are small, $\log(1 + u_{w_0 j})$ can be approximated by $u_{w_0 j}$. Thus another way to write $\log x_1$

$$\log x_1 \approx \log w_1 + u_1, \tag{4.5}$$

where $u_1 = \sum_{j=1}^{m} u_{w_0 j}$ follows normal distribution with mean zero and variance $m\sigma_u^2$, i.e. $u_1 \sim N(0, m\sigma_u^2)$. Therefore on average the target top concentration is attained, since $E(\log x_1) \approx \log w_1$. Also from (4.5), a general model for all the actual concentrations can be given by $\log x_t \approx \log w_t + \sum_{l=1}^{t} u_l$, $t = 1, 2, \ldots, n$, and the covariance between the tth and $t'th$ concentrations, $\operatorname{cov}(\log x_t, \log x_{t'}) \approx \sigma_u^2 (\min(t, t') + (m - 1))$, $t, t' = 1, 2, \ldots, n$. Hence, although the actual concentrations are different from those specified by the target design, on average the desired concentrations are applied, but with increased variation and correlated structure due to DE. In Racine-Poon et al. (1991), the bioassay data was fitted using a 4-parameter logistic response model.

In their work, a homogeneous response model was assumed. However, when EIV occur, results based on applying first-order Taylor series approximations, using the delta method for the response model showed that, $E[y_t \mid w_t;\boldsymbol{\beta}] \approx f(w_t,\boldsymbol{\beta})$, $\text{var}(y_t \mid w_t;\boldsymbol{\beta}) \approx \text{var}(y_t \mid x_t;\boldsymbol{\beta}) + D$, $t = 1,2,\ldots,n$, where $\text{var}(y_t \mid x_t;\boldsymbol{\beta})$ is a function in the response variance σ_ε^2 and the number of replications of the *t*th response; D is a function in $\boldsymbol{\beta}$, the target concentrations w_t, and the variance-covariance matrix of the true concentrations x_t, including the error variance σ_u^2. Thus, the response is heterogeneous if the errors are ignored. Note that, first-order approximations are usually considered to be appropriate, when the EIV variance σ_u^2 is small. The approximated response model is estimated using Bayesian MCMC simulation approach. Pilot studies have been used to specify the priors of dilution errors variance and other model parameters. The validity of their approach relies on an appropriate specification of the required assumptions (e.g. variance and density function of the DE); otherwise accurate inferences regarding the potency are unachievable.

Rudemo et al. (1989) investigated the problem in nonlinear random effects models. These models consider the effects of the parameters of the model to be random variables drawn from a specific distribution. Different from the previously mentioned studies, they address the bias in the response by taking 2nd order Taylor approximation for the mean of the response model. However, the bias term was considered to be negligible, unless the errors are large.

Buonaccorsi and Lin (2002) considered the effect of the same DE as Racine-Poon et al. (1991) on random effects linear and quadratic response models. More errors structures were also studied. They found that the effect of errors on the analysis differs according to the structure of error assumed to generate the actual doses. For example, if the errors in the doses are independent of each other and between individuals, naive estimators of the linear model

parameters are unbiased and efficient. However, estimates of parameters from the quadratic model were found to be biased. To address the bias, approximate formulas have been obtained. This was made rather easy because of the linearity assumption they use for the response models assumed to fit the data.

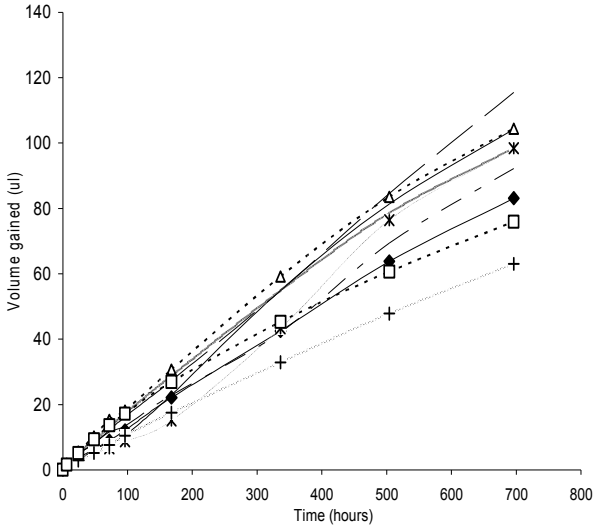
Wang and Davidian (1996) showed that only when the EIV in concentrations are normally distributed with non-zero mean, the estimates of the model parameters are biased. However, no correction method was established to adjust for such a bias.

EIV with distributional assumptions, such as, non-zero mean or non-Gaussian distribution, are common in bioassays. The following example provides evidence for that.

Example. Dawson and Donev (2007) present the results of a study on the stability of solutions for testing in bioassays when the compounds to be tested are dissolved in dimethyl sulfoxide (DMSO). DMSO is a widely used solvent because of its excellent solubilising ability, chemical inertness, high boiling, and freezing points. However, it is also highly hydrophilic and will rapidly absorb water in many possible storing conditions. This may result in reduced stock concentration, compound precipitation, crystallization, and degradation. As a result, the concentrations of compounds used in bioassays would be lower compared to those specified by the experimental design and inaccurate data would be used in a naive statistical analysis ignoring the EIV. The experiment reported by Dawson and Donev (2007) aimed to quantify the dilution of compounds as a result of the absorption of water by DMSO. Compounds from different chemical series and molecular weight bands were used. The dilution by water uptake was measured by recording the volume gain during a typical laboratory storage period. When the compounds were stored in room conditions, 11%–16% dilution was noted, while when they were stored in a fridge it was 10%–24%. Figure 4.2 shows a plot for the diluted compounds over a period of time measured in hours, in which the

compounds where stored. Different compounds are plotted in order to show how they are affected by dilution errors in different ways.

Figure 4.2 The dilution of nine compounds measured by the volume uptake over a period of around 700 hours.



Results showed that if the actual concentrations are less than the target ones, the true potency of compounds are underestimated by ignoring the errors. Therefore, since it is possible for compounds in a study to be exposed to different types of errors, the rank order of compounds is affected as a result of analysis that ignores these errors. The variance of the potency estimates is underestimated as well. This leads to wrong confidence intervals and power analysis for choosing appropriate sample size. The distribution of the resulting errors in setting the concentrations can be modelled in different ways, but a Gamma distribution seemed to suit the collected data well. See Cheng, et al. (2003) for a comprehensive study of the stability of compounds in DMSO.

Similar errors can be seen when the compounds under study have high viscosity, which causes the compounds to stick in the disposing pipette devices. There are also situations when the concentrations are larger than the planned ones. For example, if the pipette gets into

contact with the compounds in a plate of wells, it may cause an increase in the amounts of compounds all over the plate as the pipette transfers an excess amount in each disposit of the solution. In addition, errors in setting the concentrations could be also introduced entirely at random.

In this chapter, we study the effect of non-Gaussian errors in setting concentrations in bioassay and propose a correction method aimed at reduce the effect of the errors on the analysis. The method can also be used in other situations where non-Gaussian Berkson EIV occurs. Two error models are investigated. First, we assume the error only occurs in the top concentrations of a SDD, which would be the typical scenario wherein the DMSO is used, to obtain the initial concentration. Second, we assume that all the concentrations in the SDD are subject to independent errors. Such a scenario can occur, for example, in animal studies where each dose is prepared, prior to administration to each animal. The assumption of Gaussian errors is also investigated to show the differences between the effects. Both of the error scenarios have been examined before in Buonaccorsi and Lin (2002) and Gelman et al. (2004). However, our work expands their investigation by examining errors with Gaussian and non-Gaussian distributions. Moreover, unlike Buonaccorsi and Lin (2002), we study the effect on nonlinear dose-response model (4.1), and using SDDs.

Section 4.2 gives the statistical model and the structure of the EIV models. Section 4.3 examines the effect of those errors on the analysis of bioassay results by conducting a large number of Monte Carlo simulations. This reveals interesting features of the effect of errors in setting the concentrations. Section 4.4 presents an adjustment approach for the effect of errors on the analysis of data from bioassay experiments. The approach extends the SIMEX method to make it possible to use when the errors are of Berkson's type. A comparison between the performance of the proposed approach and the adjustment approaches in Chapter 3 (RC and WRC) is also presented to develop possible scenarios, in which one approach will perform

better than the others.

4.1.3 Statistical Analysis of Data from Bioassays

In a typical bioassay study, a response of interest, say *y*, is obtained at different doses; say *w*, of a studied compound. To interpret the dose-response relation, customarily the so called Hill equation or 4-parameter logistic model is given by;

$$E[y_t] = f(w_t, \boldsymbol{\beta}) = \beta_3 + \frac{\beta_4 - \beta_3}{1 + \left(\frac{w_t}{10^{\beta_1}}\right)^{\beta_2}}, \quad t = 1,2,\ldots,n \tag{4.1}$$

In equation (4.1), $E[y_t]$ is the expected response corresponding to dose w_t, $\boldsymbol{\beta}$ is a vector of model parameters giving by $\boldsymbol{\beta} = (\beta_1, \beta_2, \beta_3, \beta_4)^T$, β_1 is the logarithm to base 10 of the dose denoted by IC_{50}, the dose required to achieve a response half way between the maximum response β_4, and minimum response β_3. Note that, β_3 and β_4 are obtained by setting two controlled doses (commonly known as the negative and positive doses, respectively). Depending on the study, the IC_{50} could also be named EC_{50}. We use the same notation IC_{50} through out the text to avoid confusion. The parameter β_2 is called the Hill slope. Additive independent errors of measuring the response are often assumed. However, there are also cases when these assumptions have to be relaxed.

Some of the model parameters in (4.1) are estimated using common sense or scientific knowledge. In fact the majority of bioassays use model (4.1) motivated by knowledge about the underlying biology in dose-response studies. For example, the Hill slope is known to be usually equal or close to 1 (or -1 if the response increases with the dose). The values of β_3 and β_4 are known approximately prior to the data analysis, as they are not dependent on the effect of the studied compounds. All this simplifies the nonlinear regression problem. Primarily, the interest is in estimating β_1, as it provides guidance about the potency of a studied compound.

Small values of β_1 indicate high potency. When the most potent compound amongst several compounds has to be chosen, the task reduces to comparing the corresponding estimates of β_1 for each of them.

When carrying out the statistical analysis of data fitted with model (4.1), it is necessary to define the appropriate model assumptions. Otherwise, the results could be misleading. A description of the commonly used assumptions and methods of analysis are given below.

Model assumptions: Suppose *n* doses were used. The expectation and the variance of the *t*th response y_t corresponding to the *t*th dose w_t are;

$$E(y_t \mid w_t;\boldsymbol{\beta}) = f(w_t,\boldsymbol{\beta}), \qquad \text{var}(y_i \mid w_i;\boldsymbol{\beta}) = \sigma_\varepsilon^2, \quad t = 1,2,\ldots,n, \tag{4.2}$$

where $\boldsymbol{\beta}$ is a vector of *p* parameters and σ_ε^2 is the response variance. Model (4.2) is called the homogeneous response model because the variance of the response is assumed to be constant among the responses and independent of $f(w_t,\boldsymbol{\beta})$, $t = 1,2,\ldots,n$. If a heteroscedastic model assumption is appropriate, then the *t*th response y_t corresponding to the *t*th dose w_t, may be given by;

$$E(y_t \mid w_t;\boldsymbol{\beta}) = f(w_t,\boldsymbol{\beta}), \quad \text{var}(y_t \mid w_t;\boldsymbol{\beta}) = \sigma_\varepsilon^2 \left(h\{f(w_t,\boldsymbol{\beta}),\rho\}\right)^2, \quad t = 1,2,\ldots,n, \tag{4.3}$$

where *h* is the function that describes the form of the heterogeneity in the response, and it can depend on the mean function, as well as an extra parameter ρ.

Analysis of data: When assumption (4.2) is appropriate, NLS (explained in Chapter 1) is customarily used. For assumption (4.3), the use of appropriate weighting methods is important for the efficacy of the model's parameters.

The method IRWLS (also called the generalized least squares), can be used whenever the assumption of constant response variance is not appropriate. The direct weighted least squares analysis can be also used, however, this can be considered an ideal situation, since it requires the variance function to be exactly known and does not depend on any unknown

model or variance parameters. The following explanation of the method is taken from Davidian and Giltinan (1995).

The IRWLS approach starts by obtaining initial estimate $\hat{\boldsymbol{\beta}}_0$ (often estimated using least squares technique). To obtain estimates of the appropriate weights, any parameters in the variance function need to be estimated or assumed to be known. In the literature, there are various ways to do the estimation. A commonly used approach is the pseudolikelihood (PL) method. Assuming normally distributed responses, this approach is based on finding the variance parameters that minimise the PL function given by;

$$PL(\hat{\boldsymbol{\beta}}_0, \sigma, \rho) = \sum_{j=1}^{n} \left(\{y_j - f(x_j, \hat{\boldsymbol{\beta}}_0)\}^2 \{\sigma^2 h^2 \{f(x_j, \hat{\boldsymbol{\beta}}_0), \rho\}\}^{-1} + \log[\sigma^2 h^2 \{f(x_j, \hat{\boldsymbol{\beta}}_0), \rho\}] \right).$$

After estimating the variance parameters, the weights are estimated and used to obtain weighted least squares estimators of the model parameters. This process is iterated a sufficient number of times, to guarantee the convergence of the estimates of weights.

Other famous approaches aim to transform both sides of the response model in order to define a response with a constant variance (Box & Cox, 1964). Further information about the various ways of the analysis of heteroscedastic regression models can be found in Davidian and Giltinan (1995).

4.2 EIV in Bioassay Dose-Response Studies

The effect of ignoring the EIV in the analysis of bioassays presents one of the most difficult but vastly important statistical challenges. Comprehensive reviews of the existing theoretical results in this area are provided by Carroll et al. (2006). Certainly, the more complicated the response model used to fit the data from bioassay, the bigger the task of handling the EIV, and generalisation of results in such cases is particularly difficult. Algebraic derivations to show the effect of EIV for the very simple cases were used (see Buonaccorsi &

Lin, 2002), and more often Monte Carlo simulations are applied to obtain information about the statistical properties of the estimates of model parameters.

Here no analytical formulas for the bias of the naive estimates of the model parameters are obtained, obviously because nonlinear in parameters models with EIV, adapt an inherent complexity. Thus addressing the effects of those errors analytically might not be feasible. We will examine the effect of errors in setting the doses on the inferences of both the variance and bias of estimates of potency. The standard method of analysis for the nonlinear bioassay model, which is the nonlinear least squares approach, is applied.

Similar to Racine-Poon et al. (1991) and Dellaportas and Stephens (1995), we choose to investigate the effects of errors in concentrations on the response model (4.1), with the assumption of homogeneous response variance. Analytic descriptions for the structures of the investigated errors and their effect on the design levels of a SDD are given. Unlike Racine-Poon et al. (1991), we illustrate the analytic structure of the errors using the concentrations on the original scale rather than the logarithmic one. This helps to provide a broader description. For example, the structure of the errors in concentrations can be defined for any distributional assumptions (e.g. Gaussian and non-Gaussian errors). An additional advantage of our approach over all the early work is that we explain the properties of the errors for any magnitude (small, medium to large), which extends the work of Racine-Poon et al. (1991) who assume only errors with small magnitudes to occur.

i) Error Model 1

If the top concentration w_1 of a SDD is diluted with an error, even if there are no dilution errors when diluting the second and further concentrations, the actual concentrations differ from the target design points. This can be explained as follows. Let w_1 be the target top (or initial) concentration in a SDD consists of n doses, excluding the controlled points (positive and negative). If an error u_1 occurs in w_1, the actual top concentration x_1 can be given by;

$$x_1 = w_1 + w_1 u_1 , \tag{4.6}$$

where u_1 is a random error with a mean μ_u that can be equal or unequal to zero, and standard deviation σ_u, assumed to be independent of the response error ε. Model (4.6) is an EIV structure and corresponds to the case when the variance of the actual top concentration is proportional to w_1, giving by $\text{var}(x_1) = w_1^2 \sigma_u^2$, and follows the same distribution as the error u_1. The difference between equation (4.4) and equation (4.6) is then clear since in equation (4.6) the top concentration is dispensed in only one step, with an error u_1. In SDD, u_1 is propagated through the design concentrations, so according to (4.6), the actual concentrations of SDD are given by

$$x_t = w_t + u_t , \qquad t = 1, 2, \ldots, n . \tag{4.7}$$

where $u_t = k^{-(t-1)} w_1 u_1$. For example, the 2nd and 3rd actual concentrations in the SDD are $x_2 = w_2 + k^{-1} w_1 u_1$ and $x_3 = w_3 + k^{-2} w_1 u_1$, respectively. Thus from (4.7), $E[x_t] = w_t + k^{-(t-1)} w_1 \mu_u$, $t = 1, 2, \ldots, n$, and

$$\text{var}(x_t \mid w_t) = k^{-2(t-1)} w_1^2 \sigma_u^2 = w_t^2 \sigma_u^2 , \qquad t = 1, 2, \ldots, n . \tag{4.8}$$

Also, for $t, t' = 1, 2, \ldots, n$, $t \neq t'$,

$$\text{cov}(x_t , x_{t'}) = k^{-[(t-1)+(t'-1)]} w_1^2 \sigma_u^2 = k^{-(t+t'-2)} w_1^2 \sigma_u^2 . \tag{4.9}$$

Therefore, according to (4.8) and (4.9), if an error occurs only in the top concentration of a SDD, the actual concentrations of the design are heteroscedastic and dependent, so are the errors in concentrations. Note that errors in vitro could occur in different ways. Depending on the case under study, u_1 may follow Gaussian or any other distribution. A way to estimate the distributional properties of u_1 is the validation data approach. In a pilot study, the target top concentration can be replicated a number of times before the real study begins. By measuring the actual top concentrations, and comparing their values with the target concentrations, σ_u^2 can be estimated.

ii) Error Model 2

Let w_t be the target concentration in a SDD of n concentrations. Assume a separate dilution is made for each animal. If an error u_t occurs in w_t, the actual concentration x_t can be given by;

$$x_t = w_t + w_t u_t , \qquad t = 1, 2, \ldots, n , \tag{4.10}$$

where u_t, $t = 1, 2, \ldots, n$, are identically independently distributed random errors with a mean μ_u that could be equal or unequal to zero and a common standard deviation σ_u, assumed to be independent of the response error ε_t. Thus, $E[x_t] = w_t + w_t \mu_u$, $t = 1, 2, \ldots, n$, and

$$\text{var}(x_t \mid w_t) = w_t^2 \sigma_u^2 , \qquad t = 1, 2, \ldots, n . \tag{4.11}$$

Also, for $t, t' = 1, 2, \ldots, n$, $t \neq t'$,

$$\text{cov}(x_t , x_{t'}) = 0 . \tag{4.12}$$

Thus, according to (4.11) and (4.12), if independent errors occur in concentrations of a SDD, the actual concentrations of the design are heteroscedastic and independent. From model (4.8) and model (4.11), it can be seen that the variances of the actual observations from the cases of dependent and independent errors are the same. The errors again could follow different distributions.

Both error scenarios (dependent and independent errors) could have serious effects on the analysis of data from bioassay. The following simulation-based approach shows their effect on the analysis.

4.2.1 Monte Carlo Simulations for the Effect of Dependent and Independent Errors in Concentrations

In the absence of errors in concentrations, the fitted response model can be given by (4.1). However, when the target and the actual concentrations differ, the latter generates the response;

$$y_t = f(x_t, \boldsymbol{\beta}) = \beta_3 + \frac{\beta_4 - \beta_3}{1 + \left(\frac{x_t}{10^{\beta_1}} \right)^{\beta_2}} + \varepsilon_t, \quad t = 1, 2, \ldots, n, \tag{4.13}$$

where $\varepsilon_t \sim N(0, \sigma_\varepsilon^2)$. Often the actual concentrations are unknown, otherwise they could be used to fit model (4.13), and potential errors are likely to have negligible effect on the analysis of the data.

Generally, estimating the parameters of model (4.13) is difficult. For example, a moderate to large variability in the bioassay or a bad choice of initial values for the estimates of the model parameters, often creates computational problems. Here we use Monte Carlo simulations to evaluate all features of interest, however, careful considerations to the simulation settings is made to avoid these problems. We simulate a bioassay study that targets the examination of the potency of a certain compound.

The aim is to examine the estimation of β_1 in model (4.13). We do not examine the other model parameters (β_2, β_3, and β_4) since typical bioassay studies are mainly interested in identifying β_1. However, in one of the following simulation studies, we obtain their values just to understand the effect of the errors on the general inferences of model (4.13).

For the purpose of simulations, the model parameters are set to $\varepsilon \sim N(0, 0.01)$, β_4=700, β_3= $0.63 \times \beta_4$-78, and β_2 = -1.0. Scientific considerations and real data were used to select the values of model parameters. The choice of the relationship between β_4 and β_3 is based on a real data set that has not been reported here. The response error is chosen to be very small compared to the errors in concentrations, so that it has negligible effects on the results. This is also similar to assuming that the response variance is heterogeneous but the amount of heterogeneity can be neglected, if the response variance is small.

Careful consideration must be taken when choosing β_1 and the design settings in the experiment. If those two are not selected in relation to each other, the effect of the design

settings will be obvious on the estimates $\hat{\beta}_1$. Such effects could be biased and inefficient estimators of β_1. Since the focus here is on interpreting the effect of the errors on the analysis, the effect of the experimental design should be minimised. Therefore, if the design is optimal (e.g. *D*-optimal), the results obtained from the analysis of data explain the effect of ignoring the errors in concentrations. However, constructing optimal designs is not the target of this study. Instead, a good design can be constructed by the fair spread of points over the design region. The designs we use to study compounds are similar to optimal designs by nature of their construction, without getting to the complexity of their development. The idea behind it, is to set the SDD in a way so that the top dose is a function in the number of doses in the design, the dilution factor, and the IC50 of the compound. The top concentration is assumed to be $w_1 = k^d \times IC_{50}$, where d is (number of doses)/2 or [(number of doses-1)/2]+1, for even or odd number of design points, respectively. Let IC_{50}=2, the number of doses in the design is 8, k=2, hence the top dose is w_1=32. The influence of the design on the statistical analysis of the data is minimised by placing the IC_{50} roughly at the middle of the doses covered by the design. According to the current settings, the design in Table 4.1 is a good design to be used in the simulated experiments, with β_1=0.301 and IC_{50}=2 in all the simulations.

The results from the simulation studies evaluate how the estimates of potency of compounds are affected by errors in setting concentrations. These effects are illustrated using the following experimental scenarios:

- Known dependent errors occur at the top concentration of a SDD.
- Normal dependent and independent errors (denote N-D and N-I, respectively).
- Gamma dependent and independent errors (denote by G-D and G-I, respectively).

Note that in practice the errors are rarely known. However, we study them here to provide better insights into their effect.

The size of the errors in concentrations has been chosen, so it covers common

experimental situations. Some aspects about the nature of the errors were also accounted for, such as, too large errors have unrecoverable effects on the analysis, and too small errors may mask their true effects in the majority of practical situations. Note that, in practice, both cases are possible to occur, for instance, compounds could be totally degraded before use.

Here the magnitude of the errors in the data is a percentage of the diluted concentration, and it differs according to the four experimental scenarios under study. Two magnitudes of error are examined: relatively small and medium to large. For the case of known dependent error u_1 occur in w_1, u_1 is a fixed percentage or error, given by 5% for relatively small errors and 10% for medium to large errors.

In case of N-D and G-D error scenarios, the values for σ_u are chosen, so as for relatively small errors, approximately 10% of the generated errors can cause a change in w_1 that exceeds 95% of its absolute value. For medium to large errors, approximately 10% of the generated errors cause a change in w_1 exceeding 90% of its absolute value. In similar way, σ_u's are chosen for the case of relatively small, and medium to large N-I and G-I error scenarios.

Large numbers of simulations were conducted to choose σ_u. All error scenarios are discussed in turn below to provide the reader with guidance about what could result from a range of possible errors in setting the concentrations. The analysis of the simulated data is carried out in two ways:

- Naive analysis to obtain an estimate $\hat{\beta}_{1,naive}$ of β_1, in which the target concentrations are used in fitting model (4.13). These concentrations are generated by an experimental design with 8 observations, dispensed by serial pipetting, with a dilution factor k=2.
- Actual analysis to obtain an estimate $\hat{\beta}_{1,actual}$ of β_1, in which the actual concentrations are used in fitting model (4.13). These concentrations are generated

using different error scenarios.

Since we use the NLS approach, initial parameter values need to be specified prior to the analysis. Here, again to reduce the bias in the results from any source of errors other than the errors in concentrations, we assume that the best guess for the initial parameters has been made by choosing these values to be very close to the true set of parameters. The results were based on 10^4 simulations. For each analysis the bias $E[\hat{\beta}_1]-\beta_1$, percentage of absolute mean relative bias $|E[\hat{\beta}_1-\beta_1]/\beta_1|\times 100$, averaged model-based standard error, and empirical Monte Carlo standard error (SE) are reported. The Monte Carlo SE represents the square root mean of the variances that would be obtained if the errors in setting the concentrations were known, a value similar to the mean of the standard errors produced in a routine statistical analysis. To provide additional interpretation for the effect on the variance estimates, the relative standard error of $\hat{\beta}_1$ is reported.

In case the errors are non-Gaussian distributed, the mean bias is not the most appropriate measure to be used, and hence we compute the median bias (the median of the estimators minus the true parameter) and the percentage of absolute relative median bias.

As mentioned before, the responses can be generated from the doses in Table 4.1, however, a choice about the positive and negative controlled doses has to be made in order to obtain the maximum and minimum responses. Their values need to be carefully selected to avoid any computational problems when fitting model (4.13). For example, if the positive and negative controlled doses are poorly chosen in the experiment, the actual analysis may produce biased estimates of parameters. To avoid such problems, we choose $w_1\times c$ and w_1/c for positive and negative controlled doses, respectively, where c is a constant which takes different values according to the case study. For the current simulation settings and parameters, the choice of $c=10^5$ was found to be appropriate since it gave negligible bias in

$\hat{\beta}_{1,actual}$. For a specific study in practice, the choice of c is not of concern to the practitioners. It is only an issue here because the data was artificially created. Researchers usually have some knowledge about the doses required to give both maximum and minimum responses.

The approach we use for analysis depends on applying the method of nonlinear least squares to fit the response model assumed for the data. It is important to point out that the method of least squares is not affected by the non-normality departure. However this is not the case if further inferences need to be developed, such as hypothesis testing and confidence intervals (Bates & Watts, 1988, pp. 24-27). Note that the validity of the simulation codes was tested by using different simulation conditions. For example, if the EIV variances are set to zero, all the approaches of analyses are supposed to have equivalent results. Moreover, by increasing the size of the EIV, a trend of enlarged effects for the EIV was expected in the results.

4.2.1.1 Known Dependent Errors at the Top Concentration of a SDD

If w_1 is affected by a known error u_1, two types of errors can occur, u_1 causes x_1 to be larger than w_1 (positive error) or u_1 causes x_1 to be less than w_1 (negative error). Thus, from model (4.6), x_1 can be given by;

$$x_1 = w_1 + w_1 u_1, \tag{4.14}$$

or

$$x_1 = w_1 - w_1 u_1, \tag{4.15}$$

where w_1 is the top dose of the SDD in Table 4.1, and the error u_1 is then propagated to the remaining doses of the design.

Now models (4.14) and (4.15) are going to be studied separately to show how different types of errors have different effects on the analysis of data. The results in Table 4.2 and 4.3 show a summary of 10^4 Monte Carlo simulations for 5% and 10% errors, respectively.

Certainly using the actual concentrations gives unbiased and efficient estimators, $\hat{\beta}_{1,actual}$.

Table 4.2 Summary of 10^4 simulations of relatively small (u_1 = 5%) errors in the top concentration for the actual and naive estimators. Simulation parameters are IC50=2, β_1=0.301, and $\sigma_\varepsilon^2 \sim N(0, 0.01)$.

Error size	Measures	Actual	Naive
+5%	Mean bias	-9.068×10^{-5}	-2.128×10^{-2}
	|Mean relative bias|×100	3.012×10^{-2}	7.069
	Averaged model-based SE	4.554×10^{-4}	4.554×10^{-4}
	Monte Carlo SE	4.826×10^{-4}	2.129×10^{-2}
	Relative SE	0.944	2.139×10^{-2}
Error size	Measures	Actual	Naive
-5%	Mean bias	-6.689×10^{-5}	2.221×10^{-2}
	|Mean relative bias|×100	2.222×10^{-2}	7.378
	Averaged model-based SE	4.561×10^{-4}	4.561×10^{-4}
	Monte Carlo SE	4.797×10^{-4}	2.222×10^{-2}
	Relative SE	0.951	2.053×10^{-2}

Table 4.3 Summary of 10^4 simulations of relatively large errors (u_1 = 10%) in the top concentration for the actual and naive estimators. Simulation parameters are IC50=2, β_1=0.301, and $\sigma_\varepsilon^2 \sim N(0, 0.01)$.

Error size	Measures	Actual	Naive
+10%	Mean bias	-1.020×10^{-4}	-4.149×10^{-2}
	|Mean relative bias|×100	3.389×10^{-2}	13.78
	Averaged model-based SE	4.550×10^{-4}	4.550×10^{-4}
	Monte Carlo SE	4.844×10^{-4}	4.150×10^{-2}
	Relative SE	0.939	1.096×10^{-2}
Error size		Actual	Naive
-10%	Mean bias	-5.433×10^{-5}	4.570×10^{-2}
	|Mean relative bias|×100	1.805×10^{-2}	15.18
	Averaged model-based SE	4.564×10^{-4}	4.564×10^{-4}
	Monte Carlo SE	4.785×10^{-4}	4.571×10^{-2}
	Relative SE	0.954	9.986×10^{-3}

The Monte Carlo SE of $\hat{\beta}_{1,naive}$ is the correct figure and the true variability to be used in subsequent statistical inferential calculations. Table 4.2 shows it is 2.129×10^{-2}, hence larger than the one that would be used if the errors in setting the concentrations were ignored, i.e. 4.554×10^{-4}. Thus the true variability is severely underestimated since the naive analysis ignores the inaccuracies in the concentration levels, in fact the average model-based SEs from the actual and naive approaches are the same. The SE is an increasing function in the magnitude of u_1. For example, the relative SE of $\hat{\beta}_{1,naive}$ from +5% and +10% error is

2.139×10^{-2} and 1.096×10^{-2}. So larger errors lead to a more severe underestimation of the true standard error of $\hat{\beta}_{1,naive}$.

The relative SE from the actual analysis is 0.944 at +5%. One may expect that more accurate estimates of the Monte Carlo SE of $\hat{\beta}_{1,actual}$ should have been obtained, however the estimates of the standard errors of the NLS estimates are only approximations to the true ones (see Section 4.2.2).

The naive estimator $\hat{\beta}_{1,naive}$ is biased even when small +5% or -5% errors occur. We can also notice that since the bias is negative at +5% errors, the naive estimator $\hat{\beta}_{1,naive}$ underestimates the statistical parameter but overestimate the true potency. On the other hand, the true potency is underestimated by ignoring -5% errors. Note that, the smaller the potency estimator of a compound, the more potent the compound is, hence from +5% and -5% errors, the true potencies are actually overestimated and underestimated biologically.

The bias is an increasing function in the magnitude of u_1. For example, the mean bias in $\hat{\beta}_{1,naive}$ from +5% and +10% errors are -2.128×10^{-2} and -4.149×10^{-2}, respectively. The percentages of absolute mean relative bias from +5% and +10% are approximately 7.069% and 13.78%, respectively.

The values of the mean bias from $+u_1$ and $-u_1$ are not equal but they are very close. For example, the mean bias from +10% and -10% errors are -4.149×10^{-2} and 4.570×10^{-2}, respectively. So we can expect that if u_1 follows a symmetrical distribution (e.g. normal), asymptotically the bias in $\hat{\beta}_{1,naive}$ could be relatively small. This is going to be verified by studying the case of N-D and N-I errors. In general, these results are important since they do not depend on any distributional assumptions about the errors in concentrations. So an idea about the effect of the errors can be developed, in case the distribution of the errors is

unknown. Other model parameters were seen to be unaffected by the errors. For instance, the mean bias in β_2, β_3, and β_4 from +5% and -5% errors in w_{top} are approximately the same when using the naive and actual approaches.

Remember that the assumption of known EIV rarely holds in practice and we examine it here only to have a primer idea about the effect of the errors in various situations. In the following simulation scenarios, the errors are assumed to have a known statistical distribution, which is an assumption that holds more to various practical situations. If a compound is tested on different occasions, the errors in setting the concentrations are most likely to be randomly distributed and not fixed values. The parameters of these distributions could be estimated from historical data or pilot studies.

4.2.1.2 N-D Errors

Assume the errors are dependent and normally distributed with mean zero and variance σ_u^2, i.e. $u_1 \sim N(0, \sigma_u^2)$, the actual top concentration x_1 is given by model (4.14), where $E(x_1) = w_1$ and $\text{var}(x_1) = w_1^2 \sigma_u^2$. For relatively small and medium to large errors, σ_u is set to 0.031 and 0.062, respectively. Table 4.4 summarises the results of 10^4 Monte Carlo simulations.

From Table 4.4, the mean bias and percentage of absolute mean relative bias in $\hat{\beta}_{1,naive}$, when both σ_u is 0.031 and 0.062, are very small or negligible. The bias was expected to increase with the error size. By looking at the bias figures in Table 4.4, it may seem like there is no relation between the bias and σ_u. However, on other simulation trials, we have assumed a larger range of values for σ_{u_1}, and as a result, a monotonic relation was found between the absolute bias in $\hat{\beta}_{1,naive}$ and σ_{u_1}. Therefore, the estimates here are unbiased.

Table 4.4 Summary of 10^4 simulations of small (σ_u=0.031) and medium to large (σ_u=0.062) N-D errors for the actual and naive estimators. Simulation parameters are IC50=2, β_1=0.301, and $\sigma_\varepsilon^2 \sim N(0, 0.01)$.

Error size	Measures	Actual	Naive
σ_u=0.031	Mean bias	-8.230×10^{-5}	-1.885×10^{-4}
	|Mean relative bias|×100	2.734×10^{-2}	6.261×10^{-2}
	Averaged model-based SE	4.569×10^{-4}	4.569×10^{-4}
	MC SE	4.845×10^{-4}	1.328×10^{-2}
	Relative SE	0.943	3.441×10^{-2}
Error size	Measures	Actual	Naive
σ_u=0.062	Mean bias	-8.233×10^{-5}	1.122×10^{-4}
	|Mean relative bias|×100	2.735×10^{-2}	3.727×10^{-2}
	Averaged model-based SE	4.569×10^{-4}	4.569×10^{-4}
	MC SE	4.846×10^{-4}	2.661×10^{-2}
	Relative SE	0.943	1.717×10^{-2}

There is a noticeable effect on the estimates of the variance of $\hat{\beta}_{1,naive}$. From the naive analysis, the relative SE's are 3.441×10^{-2} and 1.717×10^{-2}, at σ_u=0.031 and σ_u= 0.062, respectively, which shows a severe underestimation of the true SE. The estimated averaged model-based SE of $\hat{\beta}_1$ from the actual and naive analysis are the same. The variability is inflated by increasing σ_u. For example, when σ_u=0.062, the MC SE is 2.661×10^{-2}, in the mean while for σ_u=0.031, the MC SE is 1.328×10^{-2}.

4.2.1.3 N-I Errors

Assume the errors u_t, $t = 1, 2, \ldots, n$, in concentrations of a SDD are independent and normally distributed with a mean zero and fixed variance σ_u^2, i.e. $u_t \sim N(0, \sigma_u^2)$. The actual concentrations x_t, $t = 1, 2, \ldots, n$, can be given by model (4.10), where $E[x_t \mid w_t] = w_t$ and $\text{var}(x_t \mid w_t) = w_t^2 \sigma_u^2$. For relatively small and medium to large errors, σ_u is set to 0.031 and 0.062, respectively. Table 4.5 summarises the results of 10^4 MC simulations. As we can see from Table 4.5, $\hat{\beta}_{1,naive}$ is asymptotically unbiased at σ_u=0.031, and it adapted a considerably small mean bias at σ_u=0.062.

Table 4.5 Summary of 10^4 simulations of small (σ_u=0.031) and medium to large (σ_u=0.062) N-I errors for the actual and naive estimators. Simulation parameters are IC50=2, β_1=0.301, and $\sigma_\varepsilon^2 \sim N(0, 0.01)$.

Error size	Measures	Actual	Naive
σ_u=0.031	Mean bias	-7.683×10^{-5}	1.267×10^{-4}
	|Mean relative bias|×100	2.552×10^{-2}	4.210×10^{-2}
	Averaged model-based SE	4.561×10^{-4}	7.694×10^{-3}
	MC SE	4.830×10^{-4}	7.846×10^{-3}
	Relative SE	0.944	0.981
Error size	Measures	Actual	Naive
σ_u=0.062	Mean bias	-7.674×10^{-5}	7.617×10^{-4}
	|Mean relative bias|×100	2.549×10^{-2}	0.253
	Averaged model-based SE	4.562×10^{-4}	1.542×10^{-2}
	MC SE	4.832×10^{-4}	1.575×10^{-2}
	Relative SE	0.944	0.979

This is made clear from the figures of percentages of absolute mean relative biases. The variability is inflated as a result of ignoring the errors in the data, and there is a monotonic relation between true variability and σ_{u_1}. For example, the MC SE is 7.846×10^{-3} and 1.575×10^{-2}, at σ_u=0.031 and σ_u=0.062, respectively.

Unlike in the case of N-D errors in concentrations of SDD, the estimated averaged model-based SE of $\hat{\beta}_1$ from the naive analysis is larger than the one from the actual analysis. For example, when σ_u=0.062, the averaged model-based standard errors of $\hat{\beta}_{1,actual}$ and $\hat{\beta}_{1,naive}$ are 4.562×10^{-4} and 1.542×10^{-2}, respectively. The relative SE of $\hat{\beta}_{1,naive}$ from both small and medium to large errors are 0.981 and 0.979, respectively. Surprisingly, the actual standard errors at both σ_u=0.031 and σ_u=0.062 seem worse than the naive ones. Again, this is because the nonlinear least squares approach, only approximates the true standard errors of the estimators. However, we still believe the actual analysis is adequately used in the comparison to evaluate the results of the naive analysis.

In the previously studied scenarios of errors (known dependent and N-D), the naive analysis showed a severe underestimation of the true variability of the naive estimates. Unlike those cases, the relative SE shows that the naive analysis did capture the effect of the errors on

the variability. Thus, when N-I errors occur in concentrations of SDD, the naive analysis can provide sufficiently valid inferences.

4.2.1.4 G-D Errors

Let x_1 be less than the target amount, and it is given by model (4.15), where u_1 follows a gamma distribution with shape and scale parameters γ and δ, respectively. The parameters settings were γ=1 and $\delta = \sigma_u$, respectively, i.e. $u_1 \sim \Gamma(1, \sigma_u)$. According to distributional properties of variables with gamma distribution (see Appendix B for more information), setting the parameters in this way produces true concentrations that follows a gamma distribution with non-zero mean $E[x_1 \mid w_1] = w_1 - w_1\sigma_u$, and variance $\text{var}(x_1 \mid w_1) = w_1^2\sigma_u^2$. These settings are more feasible in bioassay data.

Similar to equation (4.7), the rest of the concentrations in the SDD can be given by; $x_2 = w_2 - k^{-1}w_1u_1$, $x_3 = w_3 - k^{-2}w_1u_1, \ldots,$ $x_n = w_n - k^{-(n-1)}w_1u_1$, and in general, the actual concentrations can be generated by;

$$x_t = w_t - k^{-(t-1)}w_1u_1, \quad t = 1, 2, \ldots, n. \tag{4.16}$$

where x_t is a linear function in u_1, so that it also follows a gamma distribution.

To find the shape and scale parameters of the distribution of x_t, the following simple algebra can be implemented. From (4.16), it follows $E[x_t \mid w_t] = w_t - w_t\sigma_u$ and $\text{var}(x_t \mid w_t) = w_t^2\sigma_u^2$. From the distributional properties of a variable with a gamma distribution, the mean and variance of x_t can be given by, $E[x_t] = \gamma \times \delta$ and $\text{var}(x_t) = \gamma \times \delta^2$, respectively. Solving for $E[x_t]$ and $\text{var}(x_t)$ gives the shape and scale parameters, $\left[w_t - w_t\sigma_u\right]^2 \left[w_t^2\sigma_u^2\right]^{-1}$ and $w_t^2\sigma_u^2\left[w_t - w_t\sigma_u\right]^{-1}$, respectively, for the actual concentrations x_t, $t = 1, 2, \ldots, n$. The median of the gamma distribution is undefined so no closed form for its

values is given here. For relatively small and medium to large errors, σ_u take the values 0.023 and 0.044, respectively. Table 4.6, summarises the results of 10^4 MC simulations.

Table 4.6 Summary of 10^4 simulations of small (σ_u =0.023) and medium to large (σ_u =0.044) G-D errors for the actual and naive estimators. Simulation parameters are IC50=2, β_1 =0.301, and $\sigma_\varepsilon^2 \sim N(0, 0.01)$.

Error size	Measures	Actual	Naive
σ_u =0.023	Median bias	-6.687×10^{-5}	6.887×10^{-3}
	Mean bias	-6.687×10^{-5}	1.009×10^{-2}
	|Median relative bias|×100	2.222×10^{-2}	2.288
	|Mean relative bias|×100	2.222×10^{-2}	3.352
	Averaged model-based SE	4.555×10^{-4}	4.555×10^{-4}
	MC SE	4.763×10^{-4}	1.459×10^{-2}
	Relative SE	0.956	3.122×10^{-2}
Error size	Measures	Actual	Naive
σ_u =0.044	Median bias	-6.166×10^{-5}	1.332×10^{-2}
	Mean bias	-6.166×10^{-5}	1.983×10^{-2}
	|Median relative bias|×100	2.049×10^{-2}	4.319
	|Mean relative bias|×100	2.049×10^{-2}	6.645
	Averaged model-based SE	4.556×10^{-4}	4.556×10^{-4}
	MC SE	4.757×10^{-4}	2.904×10^{-2}
	Relative SE	0.958	1.569×10^{-2}

From Table 4.6, we conclude some of similarities and differences between the naive analysis of bioassay data with N-D and G-D errors. Unlike the case of N-D errors, here the estimators were found to be biased. For example, at σ_u =0.044, the median and mean biases are 1.332×10^{-2} and 1.983×10^{-2}, respectively. It seems also there is a monotonic relation between the biases and σ_u.

The effect on the averaged model-based SE is approximately the same. Perhaps it is not surprising that the estimates adapted larger biases when the non-Gaussian errors with non-zero mean occur since the measurements are biased from the target doses. This result was also pointed out by Wang (2006) for the case of classical errors.

4.2.1.5 G-I Errors

If independent errors occur in each concentration and the actual concentrations are less than the target amounts, model (4.10) can be written by;

$$x_t = w_t - w_t u_t, \qquad t = 1, 2, \ldots, n. \tag{4.17}$$

Assume the errors u_t follow a gamma distribution with γ and δ parameters 1 and σ_u, respectively, i.e. $u_t \sim \Gamma(1, \sigma_u)$. We here assume for simplicity that the errors occurring in different concentrations have a constant standard deviation σ_u. Consequently, the actual concentrations follow a gamma distribution with mean $E[x_t] = w_t - w_t\sigma_u$ and variance $\text{var}(x_t) = w_t^2\sigma_u^2$, shape and scale parameters $[w_t - w_t\sigma_u]^2 \left[w_t^2\sigma_u^2\right]^{-1}$ and $w_t^2\sigma_u^2[w_t - w_t\sigma_u]^{-1}$, respectively. Table 4.7 summarises the results of 10^4 Monte Carlo simulations.

Table 4.7 Summary of 10^4 simulations of small (σ_u=0.023) and medium to large (σ_u=0.044) G-I errors for the actual and naive estimators. Simulation parameters are IC50=2, β_1=0.301, and $\sigma_\varepsilon^2 \sim N(0, 0.01)$.

Error size	Measures	Actual	Naive
σ_u=0.023	Median bias	-6.964×10^{-5}	8.963×10^{-3}
	Mean bias	-6.800×10^{-5}	1.016×10^{-2}
	|Median relative bias|×100	2.313×10^{-2}	2.978
	|Mean relative bias|×100	2.259×10^{-2}	3.375
	Averaged model-based SE	4.588×10^{-4}	5.533×10^{-3}
	MC SE	4.793×10^{-4}	1.192×10^{-2}
	Relative SE	0.957	0.464
Error size	Measures	Actual	Naive
σ_u=0.044	Median bias	-6.276×10^{-5}	1.740×10^{-2}
	Mean bias	-6.383×10^{-5}	1.988×10^{-2}
	|Median relative bias|×100	2.085×10^{-2}	5.781
	|Mean relative bias|×100	2.120×10^{-2}	6.603
	Averaged model-based SE	4.610×10^{-4}	1.103×10^{-2}
	MC SE	4.806×10^{-4}	2.360×10^{-2}
	Relative SE	0.959	0.467

In Table 4.7, the relative SE at σ_u=0.044 and σ_u=0.023, from the naive analysis are 0.464 and 0.471, respectively. Therefore, the true variability is underestimated. The naive estimates were also biased with relative medians of 2.978 and 5.808, at σ_u=0.044 and σ_u=0.023, respectively.

4.2.2 Discussion

In the simulation studies, we examined the effect of different types of error scenarios, on the naive analysis of a bioassay experiment. The effects of EIV may have a wide range of

implications on the results. Each error scenario affects the results differently. In the cases of G-D and G-I, the estimates of potencies were found to be biased. In the cases of N-D and N-I errors, the estimators were found asymptotically unbiased particularly with small errors. The bias in the estimates is determined by the error distributional assumptions. Thus, when the distribution of the errors is asymmetric with nonzero mean, the bias was seen to be significant. When the errors follow a normal distribution with zero mean and relatively small to medium variance (since the bias increases with very large EIV), the naive estimator of the potency is unbiased. Therefore, the results from the cases of N-D and N-I errors support those found by most of the previous studies, such as, Racine-Poon et al. (1991).

In the same way the results of further inferences will vary according to the error type. The estimates of the true variability of $\hat{\beta}_{1,naive}$ have been severely underestimated in cases where the errors are dependent. On the other hand, independent errors lead to more satisfactory inferences about β_1. Thus our results extend findings by Buonaccorsi and Lin (2002), since they examined different error assumption but only in a class of linear models with Gaussian distributed errors.

The size of the errors in concentrations also has an important effect on the bioassay results. For example, we found an increasing monotonic relationship, across all the error types, between the severity of underestimation of the true variability, and the size of errors in doses.

According to the results of the previous simulation studies, G-D and G-I errors have been found to produce biased estimates of β_1 and variance estimates. In the following section, we discuss the possible correction methods for the N-D, N-I, G-D, and G-I error scenarios and propose a method to correct for the effect of asymmetric errors in bioassays.

4.3 Adjusting for G-D and G-I Errors in Concentrations of SDD

Previous studies focused on adjusting the estimates of variability as a result of investigating the effect of normally distributed errors on the analysis. We believe that when D-N errors occur, the same approaches of analysis as those in Racine-Poon et al. (1991) can be applied, for example, Bayesian inferences and maximum likelihood approaches. The case of N-I has been shown here to give accurate bioassay results, hence we do not discuss an adjustment approach for such type of errors. We will be concerned with the effects on both the bias and the variance estimates of data with asymmetric errors. This has not been examined before, so the adjustment approach gives an unconventional way of analysing bioassay EIV models.

The approach SIMEX is commonly chosen as a bias correction method due to its successful use in a wide range of applications. It is a simulation-based approach, and therefore it needs to be modified according to the error structure under study. The method is defined to be "ideally suited to problems with additive measurement error, and more generally to any problem in which the measurement error generating process can be imitated on a computer via Monte Carlo methods" (Carroll et al., 2006, p. 97).

Here we propose an approach to correct for the effect of EIV based on SIMEX method and call it BSIMEX. It can be applied under the assumption of asymmetric Berkson errors in the design variables. The BSIMEX estimator is obtained and then compared with the estimators from the actual and naive approaches using the standard nonlinear least squares approach. The robustness of the approach is also examined against misspecified error assumptions. Moreover, a comparison between the performance of BSIMEX and the proposed regression calibration approaches in Chapter 3 is presented.

4.3.1 BSIMEX for G-D Errors

The approach BSIMEX is appropriate for any error scenarios wherein the Berkson errors follow asymmetric distribution. The basic concept behind the approach relies in the simulation step in the original approach SIMEX.

In SIMEX the errors are randomly generated and added to the observed variables (contaminated with errors). However, in BSIMEX randomly generated errors are either added or subtracted from the value of the variables specified by experimental design. For example, in the case of heteroscedastic dependent errors with asymmetric distribution, wherein the actual concentrations are less than the target ones, the generated errors will be added to the target design points. These errors are increasing by a factor λ. The relation between the errors and the estimators is fitted and it is extrapolated back to the actual scenario, which is in the opposite direction of the naive and the artificially generated data. Models (2.4), (2.5), and (2.6) can be used to describe this relation. Similar to SIMEX, the approach requires some information on the moments of the distribution of the EIV.

To calculate the variance of BSIMEX, we use the method of the simulation-extrapolation information variance estimation (Stefanski & Cook, 1995). In Chapter 2, Section 2.3.2, a full review on the approach can be found. Other methods to compute the variance of SIMEX have not been examined.

4.3.2 Simulation Example

Design Choice and Simulation Parameters: A Monte Carlo simulation was conducted to examine a typical situation in practice wherein BSIMEX estimator can be applied. The target of the simulation is to examine the adjusting effect of BSIMEX on the bias of the naive estimators, as a result of small G-D EIV. In the simulation, the multiplicative factor λ was set to 0,0.5,1,1.5, and 2, and B=1500 simulated samples are generated. We choose the number of simulated samples in the simulation step of SIMEX to be large, in order to guarantee the

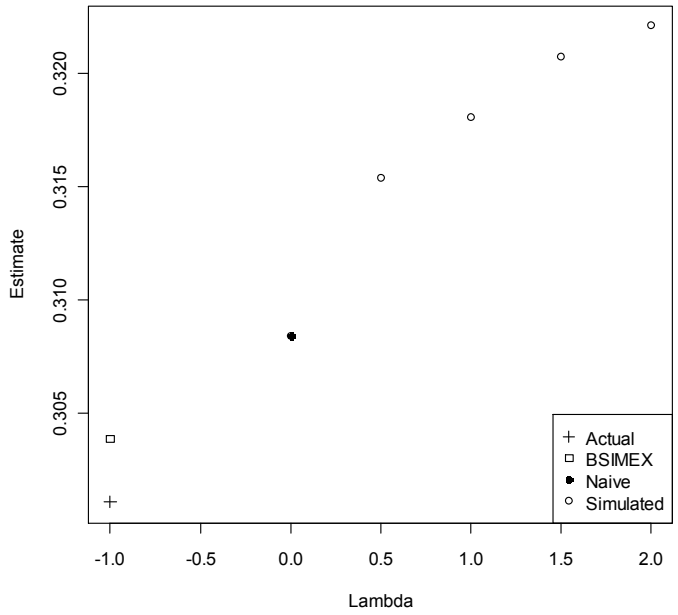
minimum amount of variability in the simulation as possible, as it can be achieved computationally. If the approach would be applied in practice, any number of *B* samples can be used. In our example, we found 100, 500, and 1000 are all possible values for *B*. For the current simulation settings, the single run consume around one minute. The SIMEX method used the NLS approach to estimate the parameters in the simulation step. The actual concentrations are generated by model (4.16).

In the simulation step, *B* new values $x^*_{b,t}(\lambda)$ are generated for each λ, by using $x^*_{b,t}(\lambda) = w_t + k^{-(t-1)}\lambda^{1/2} w_1 u_{b,1}$, $t = 1,2,\ldots,n$, $b = 1,2,\ldots,B$. Other simulation parameters and settings are same to those that have been used to illustrate the effects of G-D errors. The SDD is the design in Table 4.1, where λ is the multiplication factor, $u_{b,1}$, $b = 1,2,\ldots,B$ are identically independent random errors, simulated from gamma distribution with variance σ_u^2, the generated values $x^*_{b,t}(\lambda)$, $t = 1,2,\ldots,n$, $b = 1,2,\ldots,B$, are constantly larger than the target concentrations, and have the same distribution of the errors $u_{b,1}$.

In each *b*, $b = 1,2,\ldots,B$, the estimates $\hat{\boldsymbol{\beta}}(\lambda)$, for each λ, will be estimated from the data and averaged over *B*. Assume that $\lambda \in [0,2]$, if $\lambda = 0$, then $x^*_{b,t}(0) = w_t$, which is the naive case, wherein we assume $\text{var}(x_t \mid w_t)$ is zero. Similar to the extrapolation step in SIMEX, the parameter vector $\hat{\boldsymbol{\beta}}(\lambda)$ is modelled as a function in λ. The relation is then extrapolated by setting $\lambda = -1$, so a BSIMEX estimator $\hat{\boldsymbol{\beta}}_{bsimex}$ is obtained.

Simulation Results: Figure 4.3 shows an evidence of the improvement of the BSIMEX estimator over the naive one. The actual estimate is also shown for the purpose of comparisons.

Figure 4.3 A comparison between the actual, naive, and SIMEX estimators.



In the plot the horizontal axis is the multiplicative factor which controls the increasing amounts of the error variance verses the vertical axis which represents the values of the estimators from the actual, SIMEX and naive approaches. It can be noticed that as the error variance increases, larger biases in the nonlinear least squares estimates are found from adding positive errors to the design points, causing the potency estimates to be overestimated.

4.3.3 Simulation Example

Design Choice and Simulation Parameters: A Monte Carlo simulation was conducted to examine the asymptotic properties of the BSIMEX estimator under the assumption of G-D EIV. For 10^4 simulations, each simulation used the same parameters settings as in Section 4.3.2. The multiplicative factor λ, was set to 0,0.5,1,1.5, and 2, and B=1500 simulated samples were generated. The 10^4 simulations consume around 24×7 hours. Again the SIMEX method used the NLS approach to estimate the parameters in the simulation step. The actual concentrations are generated by model (4.16).

The results of 10^4 simulations are given in Table 4.8. They are similar to the one in Table 4.6, only the BSIMEX approach is applied and compared with the actual and naive approaches. Both assumptions of small ($\sigma_u = 0.023$) and medium to large ($\sigma_u = 0.044$) errors in top concentration were examined. The target here is to improve the naive analysis of β_1.

For the purpose of comparison linear, nonlinear and quadratic extrapolation functions were applied. A rank approach was used to determine which function obtain the worst and the best estimates of β_1, since plotting the relation between $\hat{\boldsymbol{\beta}}(\lambda)$ and λ, was not sufficient to identify an appropriate extrapolation choice. The approach is based on ranking the estimators according to their differences from β_1, and then computing the percentages of these ranks over 10^4 simulations.

Simulation Results: In terms of the appropriate extrapolation function for BSIMEX estimator, results showed that both at $\sigma_u = 0.023$ and $\sigma_u = 0.044$, the best estimator was linear BSIMEX with (36.89%) and (36.44%), respectively. The quadratic BSIMEX was second with (25.16%) and (26.23%), followed by the naive estimator and nonlinear BSIMEX, respectively. As regards to the worst estimator, at $\sigma_u = 0.023$ and $\sigma_u = 0.044$, the quadratic BSIMEX was the worst in the simulation runs by (54.36%) and (53.37%), respectively. The naive estimator came second with (45.64%) and (46.63%), respectively. The linear and nonlinear BSIMEX were not the worst estimators for both types of error.

Thus, all the approaches of the analysis produced an adequate estimator (that is least biased) in some trials, but only the naive estimator and the estimator when using the quadratic extrapolation function, interchangeably took the place of the worst estimates of β_1. Therefore, the estimators from the linear and nonlinear functions are good candidates.

We then considered making the choice of the best extrapolation function based on the variance estimates. That is, the desirable extrapolation function produces an estimate $\hat{\beta}_{1,bsimex}$

with the least variance. However, we found no significance differences between the variance of linear, quadratic and nonlinear BSIMEX. Thus in terms of the best estimator for the current case study, linear BSIMEX showed more convincing results.

In Table 4.8, only the simulation results of the BSIMEX estimator obtained using the linear extrapolation function is reported. These results show that BSIMEX produces better estimates than the naive approach that ignores the errors in concentrations.

Table 4.8 Summary of 10^4 simulations of small (σ_u=0.023) and medium to large (σ_u=0.044) G-D errors for the actual, naive and BSIMEX estimators. Simulation parameters are IC_{50}=2, β_1=0.301, and $\varepsilon \sim N(0, 0.01)$.

Error size	Measures	Actual	Naive	BSIMEX
σ_u=0.023	Median bias	-6.687×10^{-5}	6.887×10^{-3}	2.411×10^{-3}
	Mean bias	-6.687×10^{-5}	1.009×10^{-2}	5.615×10^{-3}
	|Median relative bias|×100	2.222×10^{-2}	2.288	0.801
	|Mean relative bias|×100	2.222×10^{-2}	3.352	1.865
	Averaged model-based SE	4.555×10^{-4}	4.555×10^{-4}	9.305×10^{-3}
	MC SE	4.763×10^{-4}	1.459×10^{-2}	1.194×10^{-2}
	Relative SE	0.956	3.122×10^{-2}	0.779
Error size	Measures	Actual	Naive	BSIMEX
σ_u=0.044	Median bias	-6.166×10^{-5}	1.332×10^{-2}	5.111×10^{-3}
	Mean bias	-6.166×10^{-5}	1.983×10^{-2}	1.160×10^{-2}
	|Median relative bias|×100	2.049×10^{-2}	4.425	1.698
	|Mean relative bias|×100	2.049×10^{-2}	6.588	5.316
	Averaged model-based SE	4.556×10^{-4}	4.556×10^{-4}	1.678×10^{-2}
	MC SE	4.757×10^{-4}	2.904×10^{-2}	2.417×10^{-2}
	Relative SE	0.958	1.569×10^{-2}	0.694

At σ_u=0.023, the percentage of median relative bias $\hat{\beta}_{1,bsimex}$ is 0.801%, and the percentage of median relative bias in $\hat{\beta}_{1,naive}$ is 2.288%. Thus, on average BSIMEX reduced more than half the bias in the estimate of potency.

At σ_u=0.023, the median and mean biases of BSIMEX estimator are 2.411×10^{-3} and 5.615×10^{-3}, respectively, which shows that the BSIMEX reduced the bias but not eliminated it from the analysis. A possible reason for that is because the extrapolation function was only chosen approximately; hence the correction of bias is made in an approximate way. Similar results were found when comparing the percentages of the relative mean bias in the estimates

of potency from both the naive and BSIMEX approaches. These results also hold at σ_u =0.044.

BSIMEX gives a notable improvement in estimating the true variability. The naive analysis severely underestimates the true SE with a relative SE of 3.122×10^{-2} at σ_u = 0.023, and 1.569×10^{-2} at σ_u = 0.044. BSIMEX in the mean while gives a relative SE of 0.779 at σ_u =0.023, and 0.694 at σ_u =0.044. The efficiency of $\hat{\beta}_{1,bsimex}$ is affected by increasing the error size, which explains why the relative SE was dropped down at σ_u =0.044. The variability of the BSIMEX estimator is also slightly less than the naive one. For example, at σ_u =0.023, the MC SE of $\hat{\beta}_{1,naive}$ is 1.459×10^{-2}, in the mean while the MC SE of $\hat{\beta}_{1,bsimex}$ is 1.194×10^{-2}.

The extrapolation step of BSIMEX also introduces more variability, since the chosen function to perform the extrapolation is only an approximate to the true one. The extra variability from the extrapolation step is causing an inflated SE of BSIMEX estimator, but it is mostly accounted for when estimating the variance. When the errors are large, the extrapolation step is the largest, which introduces more variability to BSIMEX's estimator (since the relative SEs are not as large as the actual analysis). However, BSIMEX still produces estimators with slightly less SEs than the naive approach and more efficient estimators.

To study the distributional properties of the actual, naive, and BSIMEX estimators, histograms are plotted for the data from 10^4 simulations at σ_u =0.023. These plots are given in Figures 4.4, 4.5 and 4.6, respectively. Asymptotically, the bias corrected estimator $\hat{\beta}_{1,bsimex}$, follows the distribution of the errors in the top concentrations, which is in our example gamma distribution. The naive estimator is also asymptotically gamma distributed; whereas the actual estimator follows a normal distribution as it fully accountable for the errors in the response models.

Figure 4.4 Histogram of the actual nonlinear least squares estimators.

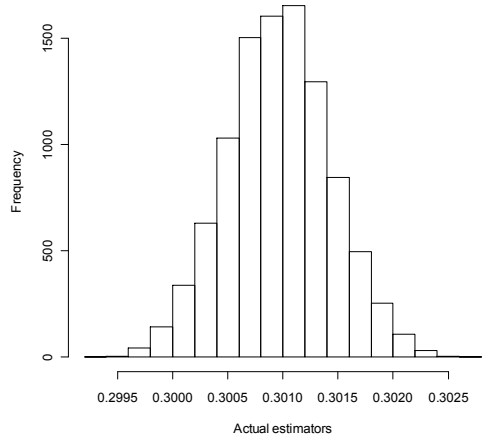


Figure 4.5 Histogram of the naive nonlinear least squares estimators.

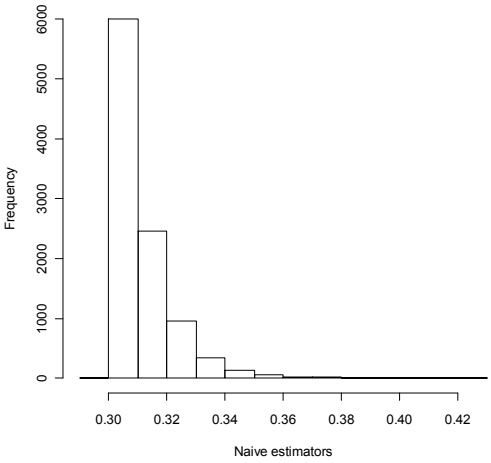
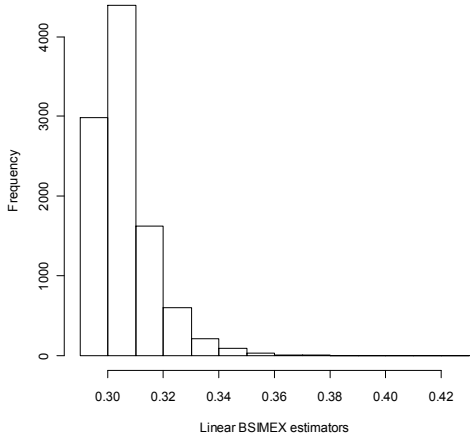


Figure 4.6 Histogram of BSIMEX estimators.



4.3.4 Robustness of BSIMEX for Misspecification of the Error Variance

We now study the robustness of BSIMEX approach under the assumption of misspecified error variance. The original SIMEX method is known to be robust for misspecification in the error variance. Assumes the case of small errors in the top concentrations, when the error variance is estimated, two possible estimation problems can occur: the error variance could be either underestimated, or overestimated. The following simulation example examines the robustness of BSIMEX for both underestimated and overestimated error variance. For the misspecified values of the error variances, a sensible range has been chosen, determined by practical considerations, and wider ranges would not be of interest but ones up to those limits are of interest.

4.3.5 Simulation Example

Design Choice and Simulation Parameters: In the simulation, the multiplicative factor λ, was set to 0,0.5,1,1.5, and 2, and B=1500 simulated samples are generated. The actual concentrations are generated by equation (4.16). Other simulation parameters and settings are similar to the ones used to illustrate the effects of G-D errors, except that only the case of relatively small errors in the concentrations with σ_u =0.023 is investigated here. Therefore, the actual and naive estimates have the same values for both underestimated and overestimated errors.

To simulate the cases of underestimated and overestimated error variance, the true variance is divided and multiplied, respectively by a constant 2. So that σ_u =0.023, is replaced by roughly 0.012 and 0.046, respectively. The results of the simulation are summarised in Table 4.9.

Simulation Results: Table 4.9 shows that the robustness of BSIMEX estimators can be affected if the error variance is misspecified. In comparison with the analysis in Table 4.8, a major reduced in the performance of BSIMEX can be noticed when the errors are underestimated.

Table 4.9 Summary of 10^4 simulations of small (σ_u=0.023) and medium to large (σ_u=0.044) G-D errors for the actual and naive and BSIMEX estimators. The errors are underestimated ($\hat{\sigma}_u$=0.012) and overestimated ($\hat{\sigma}_u$=0.046). Simulation parameters are IC50=2, β_1=0.301, and $\varepsilon \sim N(0, 0.01)$.

Error size	Measures	Actual	Naive	BSIMEX
$\hat{\sigma}_u$=0.012 Underestimated errors	Median bias	-6.687×10^{-5}	6.887×10^{-3}	4.500×10^{-3}
	Mean bias	-6.687×10^{-5}	1.009×10^{-2}	7.704×10^{-3}
	|Median relative bias|×100	2.222×10^{-2}	2.288	1.495
	|Mean relative bias|×100	2.222×10^{-2}	3.352	2.559
	Averaged model-based SE	4.555×10^{-4}	4.555×10^{-4}	5.033×10^{-3}
	MC SE	4.763×10^{-4}	1.459×10^{-2}	1.305×10^{-2}
	Relative SE	0.956	3.122×10^{-2}	0.386
Error size	Measures	Actual	Naive	BSIMEX
$\hat{\sigma}_u$=0.046 Overestimated errors	Median bias	-6.687×10^{-5}	6.887×10^{-3}	-1.676×10^{-3}
	Mean bias	-6.687×10^{-5}	1.009×10^{-2}	1.516×10^{-3}
	|Median relative bias|×100	2.222×10^{-2}	2.288	0.557
	|Mean relative bias|×100	2.222×10^{-2}	3.352	0.504
	Averaged model-based SE	4.555×10^{-4}	4.555×10^{-4}	1.745×10^{-2}
	MC SE	4.763×10^{-4}	1.459×10^{-2}	1.064×10^{-2}
	Relative SE	0.956	3.122×10^{-2}	1.640

For example, at σ_u=0.023 in Table 4.8, the percentage of median relative bias and relative SE are 0.801 and 0.779, respectively. However, in Table 4.9 and at $\hat{\sigma}_u$=0.012, the percentage of median relative bias and relative SE are 1.495 and 0.386, respectively. When σ_u is overestimated, i.e. $\hat{\sigma}_u$=0.046, the percentage of relative median bias and relative SE are 0.557 and 1.640. Hence, better results were seen when the errors are overestimated. Also the SE of $\hat{\beta}_{1,bsimex}$ when the errors are overestimated is slightly more efficient than if the errors are underestimated since the relative SE at $\hat{\sigma}_u$=0.012 and $\hat{\sigma}_u$=0.046 are 0.386 and 1.640, respectively. Thus unlike SIMEX, when the error variance is misspecified, BSIMEX fails to provide robust estimation for the EIV.

4.3.6 BSIMEX for G-I Errors

As mentioned before, the BSIMEX method is applicable for any type of Berkson errors with asymmetric distributions. To verify that, in this section BSIMEX is applied for the case of gamma independent errors in concentrations. The results in Table 4.6 showed that G-I errors in concentrations lead to biased and inefficient nonlinear least squares estimators. Therefore, BSIMEX can be used to correct for the effect of G-I errors on the analysis. The same general theory for BSIMEX with G-D errors holds for any distribution with asymmetric distribution, with slight change in the simulation step of BSIMEX, so we do not present it again.

4.3.7 Simulation Example

Design Choice and Simulation Parameters: A Monte Carlo simulation was conducted to examine the asymptotic properties of the BSIMEX estimator. In the simulation, the multiplicative factor λ, is set to 0,0.5,1,1.5, and 2, and B=1500 simulated samples were generated. The actual concentrations were generated by model (4.17). Other simulation parameters and settings are similar to those that have been used to illustrate the effects of G-I errors. We assume the errors occur in the concentrations with a fixed standard deviation σ_u. For relatively small and medium to large errors, σ_u was assumed to take the values 0.023 and 0.044, respectively, and the SDD is the design in Table 4.1.

Simulation Results: Table 4.10 summarises the results of 10^4 simulations of small and medium to large G-I errors. The naive estimator is biased at both σ_u=0.023 and σ_u=0.044. The bias increases as the error size increase. The naive also underestimates the true variability. Using the BSIMEX approach on the other hand, has produced less biased and more efficient estimators. For example, the median biases at σ_u=0.023 from both the naive and BSIMEX approaches are 8.963×10^{-3} and 4.507×10^{-3}, respectively.

The variability of BSIMEX estimator is less than the naive one, with MC SEs of 1.192×10^{-2} and 8.442×10^{-3}, for both estimators respectively. There is an obvious improvement in the efficiency at σ_u=0.023, however, at σ_u=0.044, less efficient estimators of the true variability of BSIMEX was obtained as the computed relative SE is 5.347.

Table 4.10 Summary of 10^4 simulations of small (σ_u=0.023) and medium to large (σ_u=0.044) G-I errors for the actual, naive and BSIMEX estimators. Simulation parameters are IC50=2, β_1=0.301, and $\varepsilon \sim N(0, 0.01)$.

Error size	Measures	Actual	Naive	BSIMEX
σ_u=0.023	Median bias	-6.964×10^{-5}	8.963×10^{-3}	4.507×10^{-3}
	Mean bias	-6.800×10^{-5}	1.016×10^{-2}	5.685×10^{-3}
	|Median relative bias|×100	2.313×10^{-2}	2.978	1.497
	|Mean relative bias|×100	2.259×10^{-2}	3.375	1.888
	Averaged model-based SE	4.588×10^{-4}	5.533×10^{-3}	8.333×10^{-3}
	MC SE	4.793×10^{-4}	1.192×10^{-2}	8.442×10^{-3}
	Relative SE	0.957	0.464	0.987
Error size	Measures	Actual	Naive	BSIMEX
σ_u=0.044	Median bias	-6.276×10^{-5}	1.740×10^{-2}	9.118×10^{-3}
	Mean bias	-6.383×10^{-5}	1.988×10^{-2}	1.164×10^{-2}
	|Median relative bias|×100	2.085×10^{-2}	5.781	3.029
	|Mean relative bias|×100	2.120×10^{-2}	6.603	3.867
	Averaged model-based SE	4.610×10^{-4}	1.103×10^{-2}	9.223×10^{-2}
	MC SE	4.806×10^{-4}	2.360×10^{-2}	1.725×10^{-2}
	Relative SE	0.959	0.467	5.347

Therefore, the estimation approach we use for obtaining the variance of BSIMEX was found to be inefficient when the errors are of medium to large magnitude. This issue did not arise when applying BSIMEX in case of G-D errors with both small and medium to large errors. However, this is not unexpected.

As pointed out in Chapter 2, Section 2.3.2, the method of variance estimation works well when the error size is small or with large sample sizes since the generated variability from the simulation step of SIMEX is reduced by these two. In the case of G-D errors, a random error u_1 is generated only in the top concentration of the design and is then diluted across the other concentrations. The extrapolation step of BSIMEX also introduces more variability since the chosen function to perform the extrapolation is an approximate to the true one. As mentioned before, the extra variability from the extrapolation step is causing an inflated SE of the

BSIMEX estimator, but it is accounted for when estimating the variance. Therefore, we expect the overall variability of the BSIMEX estimator to be small.

When the errors are G-I, they occur independently in each concentration of SDD. Therefore, we expect the variability in the simulation step of BSIMEX to be much larger than in the case of G-D errors, which seems to affect the efficiency of BSIMEX particularly when the errors are large. Thus, BSIMEX is suitable for any asymmetric distributed Berkson errors with small variability in the data.

4.4 Comparison between BSIMEX, RC_E, RC_A, WRC_E, and WRC_A

In Chapter 3, the regression calibration was used to deal with the effect of mixing errors on the analysis of mixture experiment. The application of the method was supported by the direct estimation in the form of the expectation of the actual variable given the design point. Particularly, when the weighted least squares approach is used in combination with the regression calibration, the results were found to be approximately unbiased and efficient and the method was named WRC. The weights are going to be the inverse of the variances of the responses at each design point. The mixing errors occur independently in the amounts of the components of a mixture. Therefore, the responses obtained from the different trials are independent that is, the off-diagonal elements in the variance-covariance matrix of the responses are zeros.

When the dilution errors occur in the doses of a bioassay experiment, two error structures have been studied here: dependent gamma dilution errors and independent gamma dilution errors. In both cases, a direct estimation for $E[x_t \mid w_t]$ was obtained, which gives an analytic way to obtain the calibrated design matrix. An empirical estimate of $E[x_t \mid w_t]$ is also possible to estimate by generating a large number of a simulated true variables and then

averaging over their values. Thus, using $E[x_t \mid w_t]$ in the regression model should produce an approximation of the true or actual analysis. The assumption of gamma errors is not necessary here. As a matter of fact, when Gaussian EIV exist with non-zero mean, the actual values are biased from the design points.

Moreover, if the distributional properties of the responses are available, the WRC approach can be seen as a solution for the effect of the dilution errors. Note that, since the working model is nonlinear in parameters, the nonlinear weighted least squares approach is used to obtain unbiased and efficient results instead of the ordinary weighted least squares in the mixture problem. However, two important distinctions between the structures of the responses, as a result of the dilution errors must be pointed out here:

- For G-D errors, the responses are correlated and heterogeneous, that is $\text{var}(y_t) \neq \sigma_\varepsilon^2$ and $\text{cov}(y_t, y_{t'}) \neq 0$, $t, t' = 1, 2, \ldots, n$. This is because the concentrations are correlated and heterogeneous.
- For G-I errors, the responses are independent and heterogeneous, that is $\text{var}(y_t) \neq \sigma_\varepsilon^2$ and $\text{cov}(y_t, y_{t'}) = 0$, $t, t' = 1, 2, \ldots, n$. This is because the concentrations are independent and heterogeneous.

Thus only when the errors are of G-I type, WRC approach is applicable since the nonlinear weighted least squares approach we apply here assumes independent responses (R CRAN, 2000). The dependency in the responses as a result of the dilution errors was addressed before by researchers, such as, Higgins et al. (1998).

We expect efficient results to be obtained if the WRC is used. On the other hand, if the BSIMEX is applied with large G-I errors, the simulation results in Section 4.3.7 showed that BSIMEX gives approximately unbiased but inefficient estimators. This can be an advantage for the approach WRC over the BSIMEX method. In the following simulation example, the

RC, WRC, and BSIMEX are compared. Comparisons between the empirical and analytic approaches RC_E /WRC_E and RC_A/WRC_A are also given. Note that, the proposed approaches are targeting the reduction of the bias in the estimators of the model parameters. Thus again, adjusting the effect of Gaussian distributed errors was not investigated here since the naive estimators are asymptotically unbiased.

- Simulation Example

Design Choice and Simulation Parameters: The same simulation settings in Section 4.3.7 is used in order to illustrate a comparison between the RC, WRC and BSIMEX. An empirical estimate of the calibrated design matrix is computed over 10^4. This number was seen as large enough to achieve convergence. The weights are also computed over 10^4 simulations. The response error variance is assumed to be known or well estimated prior to the analysis and its value is σ_ε^2=0.01.

Simulation Results: Table 4.11 shows the results of 10^4 simulations of the actual, naive, BSIMEX, RC_E, RC_A, WRC_E, and WRC_A. The approaches are distinguished according to the bias adjusted and efficiency of their estimators. The results of the actual, naive, and BSIMEX approaches have been reported before in Table 4.10, and they are reported again here to compare them with the results from the regression calibration methods.

The naive estimator was the worst between all the estimators from the other analysis; hence the adjustment methods provide an improvement over the naive approach. BSIMEX showed more efficient estimators than the other adjustment methods at σ_u=0.023, but with larger biases than those methods. However, the efficiency of BSIMEX has been reduced with larger errors in the concentrations (e.g. at σ_u=0.044). At both small and large errors, the average model-based SEs of $\hat{\beta}_{1,bsimex}$ are the largest among the adjustment methods.

The WRC performed well in terms of bias-correction and variance estimates. The RC estimators, unexpectedly though, had slightly more efficient estimates than the WRC estimators. In the meantime, the WRC estimators had less bias than the RC estimators. Note that, the mean biases of the estimators from the RC approach are less than the median biases of the estimators from the WRC method, however, since the distribution of the parameters is skewed, we only focus on the median of the estimators.

The SEs of the estimators from WRC were smaller than the ones from the RC, for example, at σ_u =0.044, the MC SEs from the RC_E and WRC_E approaches are 1.222×10^{-2} and 7.615×10^{-3}, respectively. Thus, the WRC can be recommended over the RC method, on the cost of a slight compromise in the efficiency of the estimators. Moreover, it can be seen that BSIMEX is not recommended when the errors are independent, and as an alternative, a regression calibration approach can be used to correct for the bias of the naive estimates. If an estimate of σ_ε^2 could be obtained, the WRC seems to be an appropriate replacement for BSIMEX, else RC can be used instead.

Table 4.11 Summary of 10^4 simulations of small (σ_u =0.023) and medium to large (σ_u =0.044) G-I errors for the actual, naive, WRC, and BSIMEX estimators. Simulation parameters are IC50=2, β_1 =0.301, and $\varepsilon \sim N(0, 0.01)$.

Error size	Measures	Actual	Naive	BSIMEX	RC_E	RC_A	WRC_E	WRC_A
	Median Bias	-6.964×10^{-5}	8.963×10^{-3}	4.507×10^{-3}	-1.034×10^{-3}	-1.067×10^{-3}	-3.960×10^{-4}	-4.093×10^{-4}
	Mean Bias	-6.800×10^{-5}	1.016×10^{-2}	5.685×10^{-3}	3.832×10^{-5}	5.806×10^{-6}	6.503×10^{-5}	5.165×10^{-5}
	|Median relative bias|×100	2.313×10^{-2}	2.978	1.497	0.343	0.354	0.132	0.136
σ_u =0.023	|Mean relative bias|×100	2.259×10^{-2}	3.375	1.888	1.929×10^{-3}	1.929×10^{-3}	2.160×10^{-2}	1.716×10^{-2}
	Averaged model-based SE	4.588×10^{-4}	5.533×10^{-3}	8.333×10^{-3}	5.549×10^{-3}	5.550×10^{-3}	3.456×10^{-3}	3.456×10^{-3}
	MC SE	4.793×10^{-4}	1.192×10^{-2}	8.442×10^{-3}	6.861×10^{-3}	6.087×10^{-3}	3.864×10^{-3}	3.864×10^{-3}
	Relative SE	0.957	0.464	0.987	0.912	0.912	0.894	0.894
Error size	Measures	Actual	Naive	BSIMEX	RC_E	RC_A	WRC_E	WRC_A
	Median Bias	-6.276×10^{-5}	1.740×10^{-2}	9.118×10^{-3}	-1.815×10^{-3}	-1.876×10^{-3}	-5.449×10^{-4}	-5.694×10^{-4}
	Mean Bias	-6.383×10^{-5}	1.988×10^{-2}	1.164×10^{-2}	3.937×10^{-4}	3.330×10^{-4}	4.716×10^{-4}	4.458×10^{-4}
	|Median relative bias|×100	2.085×10^{-2}	5.781	3.029	0.603	0.623	0.181	0.189
σ_u =0.044	|Mean relative bias|×100	2.120×10^{-2}	6.603	3.867	0.131	0.111	0.157	0.148
	Averaged model-based SE	4.610×10^{-4}	1.103×10^{-2}	9.223×10^{-2}	1.102×10^{-2}	1.103×10^{-2}	6.724×10^{-3}	6.724×10^{-3}
	MC SE	4.806×10^{-4}	2.360×10^{-2}	1.725×10^{-2}	1.222×10^{-2}	1.222×10^{-2}	7.615×10^{-3}	7.614×10^{-3}
	Relative SE	0.959	0.467	5.347	0.902	0.902	0.883	0.883

4.5 Summary and Discussion

We examined the effect of Berkson error using several Monte Carlo simulations of bioassay dose-response experiment. The target was to obtain valid inferences of the biological activity that has been studied. The effect on the response measurements was not studied since in the practical examples we illustrated, typically more attention is given to the potency estimates of compounds. The errors in concentrations were assumed to be heteroscedastic and independent or dependent. In the case of independent errors in concentrations of serial dilution designs, the effect of EIV on the analysis was smaller than when the errors were dependent.

We also examined the effect of symmetric and asymmetric EIV on the naive analysis. In the literature, major studies have suggested different ways to account for the effect of EIV on the variance estimates. These studies have only examined the effect of symmetric errors. We developed the Berkson simulation-extrapolation approach to correct for the effects of asymmetric errors on the analysis of data.

A comparison of Example 4.3.3 and Example 4.3.7 presented in Tables 4.8 and 4.10, respectively, show that when the EIV have non-zero expectation, the parameter of interest, logIC50, is estimated with bias. However, when the EIV are dependent this does not lead to an increase in the residual sum of squares (RSS) from the naive estimation and therefore the true variability is underestimated. As a result, the RSS from the naive analysis are identical to that of the actual analysis. Hence when a bias correction is implemented with BSIMEX, RSS closer to the true are obtained. When the EIV are independent, the RSS of the naive estimation of the model parameters is increased and estimated correctly. Furthermore, BSIMEX reduces the RSS due to the EIV but the averaged model-based standard error of the estimate is not efficient.

Results showed that the adjusted estimator is approximately unbiased and efficient. Although the variances of the estimators are underestimated when the errors are large, the approach provides a noticeable improvement over the naive analysis. The extrapolation function being used in BSIMEX influences the consistency of the estimators. In all examples that we considered, the linear and nonlinear extrapolation function gave consistent results regarding the bias. All extrapolation functions also led to approximately efficient estimates compared to the naive counterpart, though there might be situations where this is not the case. However, the consistency of the original approach SIMEX can also be affected by the wrong choice of the extrapolation function. Despite that SIMEX is one of the most used approaches for measurement error.

The assumption of heteroscedastic errors in concentrations has been used in all the simulation trials. However, BSIMEX should also work well with homoscedastic errors in concentrations. The method is supposed to be applicable for any errors with asymmetric distribution other than gamma. The robustness of BSIMEX was also investigated. Results showed that it is very important for the error variance to be known prior to the analysis.

The performance of BSIMEX was compared with the regression calibration and weighted regression calibration methods. The later approaches were proposed in Chapter 3 to correct the bias in the estimators of the commonly used linear models to fit the responses in mixture experiments. The comparison was carried out for the case when the errors are independently distributed. It was not carried out when the errors are dependent because it is clear that the regression calibration approach would be inadequate in that case as it does not account for the dependence of the observations.

BSIMEX is found to be robust for dependent and heteroscedastic Berkson EIV. The RC approaches, on the other hand, are robust for independent and heteroscedastic Berkson EIV. For example, the approach BSIMEX is inefficient when the errors are independently

distributed; however, the RC methods seem to be performs better than BSIMEX. The reason may be due to the increased amount of variability in the simulation step of BSIMEX; however, more studies can be implemented to investigate the inappropriateness of BSIMEX for independent errors. Table 4.12 summarises the comparison between the naive, Berkson simulation-extrapolation, regression calibration, weighted regression calibration approaches for dealing with N-D, N-I, G-D and G-I errors.

Table 4.12 Comparison between the naive, Berkson simulation-extrapolation, regression calibration and weighted regression calibration approaches for dealing with N-D, N-I, G-D, and G-I errors.

Error type	Naive	Berkson simulation-extrapolation	Regression calibration	Weighted regression calibration
N-D	Unbiased but severely inefficient	Not applicable (since the naive estimates are unbiased)		
N-I	Unbiased and efficient			
G-D	Biased and severely inefficient	Approximately unbiased and efficient	Not applicable (since it does not account for the correlation structure of the responses)	
G-I	Biased and moderately inefficient	Approximately unbiased and inefficient	Approximately unbiased and efficient	Approximately unbiased and efficient

5 Summary and Future Work

5.1 Summary

This thesis investigated the effect of Berkson errors in the design levels of mixture and bioassay dose-response studies. The main target was to develop a comprehensive knowledge about the possible consequences of ignoring the errors in the design variables. The target has been achieved by examining the effect of the errors, using different experimental design situations. The focus was on dependent and heteroscedastic Berkson errors, as they have been less examined in the literature of EIV.

The common assumption about Berkson errors is that they lead to no bias in the estimates of parameters. We show that this is not true when the errors are either dependent or non-Gaussian with non-zero mean. In some cases, the bias was relatively small, and hence may seem to be not important when accounted for in the analysis of the data. However, the bias is mainly a function of two components: the model parameters and variances of errors-in-variables. Thus, larger biases can occur with large values of at least one of these components. In addition, mixtures and bioassay studies are one of the vital studies where even a small bias must be quantified. After all, such studies are expensive, and run over a long period of time,

not to mention their key part in the development of products or medical treatments. Therefore, the most efficient and accurate results must be obtained. Here is a review for the contents of Chapters 2, 3, and 4.

In Chapter 1, an introduction was presented to the concept of errors-in-variables, and the different sources of such errors in practical fields of studies. Mainly, two types of errors were considered: classical and Berkson errors. In most experiments, predictors can not be measured or set exactly and an alternative measure is used. How the measurement related to the exact predictor is a question that addresses whether the error is of classical or Berkson's type. We discussed the effect of errors-in-variables on different types of statistical models. Those effects can be summarised as larger residual variance and biased least squares estimates. A summary of the most well-known adjustment techniques, mostly used to estimate the parameters of those models, was also presented. A general pattern about all the estimation methods is that each method has some advantages and disadvantages. No particular method works better than any other method in all practical situations. There are some factors that control the efficacy of an adjustment approach. These factors could be the sample size, the variances of the errors-in-variables (large or small, known or unknown), the structure of errors-in-variables (e.g. classical, Berkson, or both), the model (linear or nonlinear), and the validity of the distributional assumption, if it is required (e.g. dependent or independent heterogeneous or homogeneous errors-in-variables).

During the investigation that has been carried our here, a set of novel ideas in regards to the general problem of errors-in-variables were established. The main outcomes were recognised using, firstly, an example of some of the typical linear response models in mixture studies. Secondly, a much used nonlinear response model applied to study the relation

between the doses and responses in bioassay studies.

The study in Chapter 3 regarding the effect of mixing errors on mixture experiments identified very interesting results. Using the delta method, analytical results were developed to show the effects of those errors on the analysis of the data obtained in mixture experiments. The presented results show that ignoring the mixing errors in the design variables of linear models leads to approximately biased ordinary least squares estimators of the model parameters and overestimated variances of those estimators. The direction and size of the bias depend on many factors, but mainly on the size of the mixing error variances. As a result of the mixing errors, the model becomes approximately biased and with increased and heterogeneous variance. If the mixing error variances are known or accurately estimated, the specification of the form of the bias is a very important stage in addressing this problem because it allows for correcting for it using the regression calibration approach.

Moreover, in practice, it is mostly assumed that when mixing errors occur in the amounts of a mixture, on average the required proportions are attained. Here we proved that this is not true, and we made use of these results by combining a straightforward application of the regression calibration approach and the weighted least squares method, and called it weighted regression calibration approach. First and second-order Scheffe polynomials were used in the simulation examples, but other types of models can be used. To deal with the problem in other types of models, an empirical estimate of corrected extended design matrix was estimated as well.

However, there are some limitations with the application of the proposed approaches as they could be computationally intensive. In addition, accurate estimates of the error variances must be available through validation data, which could not be possible in some practical

situations, such as, when expensive materials are used in the study. These limitations are common in most of the correction approaches for the effect of EIV as discussed in Chapter 2. The approaches we proposed though are very easy to implement as they can be developed using both analytical and empirical methods, which is not available in most of the correction techniques, particularly in the mixture problem.

In Chapter 4, examining the effect of ignoring the errors in design levels of doses in dose-response studies also turned out to be interesting. The effects are studied by using the Hill nonlinear response model, which contains four parameters: top, bottom, slope and $\log IC_{50}$. The target was to examine the accuracy and efficiency of the estimates of the $\log IC_{50}$ parameter under different Berkson error scenarios.

We show that, different errors have different effects on the analysis. For example, unlike most of the researches of the EIV problem, where Gaussian errors are mostly assumed, we investigate the assumption of non-Gaussian (dependent and independent) errors. Non-Gaussian errors are very common in medical researches and bioassay studies, and we have shown one example of where these errors can occur. For the purpose of illustration, we assume the errors are gamma distributed. However, any other distribution could lead to similar results. The main outcomes of our work were based on comparisons between the effect of normally dependent, normally independent, gamma dependent and gamma independent errors.

The results show that ignoring gamma distributed errors lead to biased nonlinear least square estimates of the $\log IC_{50}$, and underestimated variances. The effect of dependent Gaussian errors on the analysis was also found to be serious, as the variance of the estimator of $\log IC_{50}$ is severely underestimated if the errors were ignored. This partially contradicts the results we obtained in Chapter 3, since Berkson errors were seen to cause overestimated

variances of estimators.

The dependency between the errors, whether they follow Gaussian or non-Gaussian distribution, gives a larger loss of efficiency than the case of independent errors. Therefore, we recommend the experimenter to carry on an adjustment approach, if the errors in the design variable were believed to be dependent.

To correct for the effects of the gamma errors-in-variables, we extended the SIMEX method, and called the new approach BSIMEX. The approach provides approximately unbiased and efficient estimator of $\log IC_{50}$. However, some practical limitations when applying the BSIMEX approach have to be considered. BSIMEX is only successful for one of the studied error scenarios, that is, non-Gaussian errors in design levels. This is because more consistent and efficient results were obtained, when the errors are non-Gaussian and dependent, rather than the case of non-Gaussian and independent, particularly in regards to estimating the standard errors of the estimators of model parameters.

As for the case of dependent and non-Gaussian error occuring in the top design level, the error is propagated in the design, and hence diluted. On the other hand, if independent non-Gaussian errors occur in all the concentrations, the variability is increased in the simulation step of BSIMEX, as different errors are generated at each design point. A possible solution for this is to increase the sample size in the simulation step of BSIMEX, in order to capture more of the variability in the generated data. However, this is not a guaranteed solution for more efficient estimators, as the number of simulations is an arbitrary measure.

The approach is computationally expensive and requires accurate estimator of the error variance. It adjusts approximately for the bias, as it depends on an approximate extrapolation step. In addition, compared to the regression calibration method, no analytical formulas for the

bias, in the estimators of model parameters were developed.

Even though BSIMEX can be seen to have some practical limitations, it is still worth being investigated. The approach SIMEX was developed by Cook and Stefanski (1994) to deal with classical Gaussian EIV. Therefore, extending the approach to deal with Berkson errors represents a major feature of novelty in this work. The approach is easy to implement and does not require a strong mathematical background compared to the previous work on errors in bioassays (e.g. see the adjustment approach used by Higgins et al. (1998)). It can be included also as a built-in function in R CRAN, similar to the original SIMEX. Note, in future work, it would be useful to compare between BSIMEX and the approaches presented in the literature to deal with EIV in bioassays, as it is interesting to see how these approaches will be affected by the assumption of non-Gaussian errors in the concentration levels.

The proposed approaches: weighted regression calibration and BSIMEX can be used to produce bias-corrected and efficient estimators. However, there are some differences in their performances according to the area under study. The adjustment approach we proposed in Chapter 3, that is, the weighted regression calibration, is ideally suitable when an estimate of the response variance σ_ε^2 is available, from historical data or validation studies. On the other hand, BSIMEX does not require any knowledge about σ_ε^2. In bioassays, an unbiased estimate of σ_ε^2 may not be as direct as you can get in the mixture problem. Usually the variability between the plates where the doses are disposed, and within the plates also, should be considered, since these studies incorporate more sources of errors. Moreover, there is always a question of whether the expectation of the actual concentration given the target one is appropriate to be applied when the distribution of the unobserved variable is non-Gaussian and sharply skewed. Thus, over all, the choice to apply BSIMEX over the regression calibration

was more sensible, when dealing with errors-in-variables in bioassay studies.

Using a simulated experiment, we compared between the Berkson simulation-extrapolation, the regression calibration (empirically and analytically) and weighted regression calibration (empirically and analytically). The comparison was useful as it addresses the various situations or error structure in which the proposed approaches adjust differently for the effect of the errors-in-variables.

The estimators from the regression calibration approaches were found approximately unbiased and more efficient than the BSIMEX's estimator, in cases where the errors occur independently in the diluted concentrations. An interesting feature was also revealed, that is both the regression calibration and weighted regression calibration, adjust approximately in an equal way for the errors-in-variables, however, the latter approach seems to perform slightly better. Thus, if no estimates for the errors in the responses are available, the regression calibration method is sufficient to estimate for the model parameters.

BSIMEX has its own merit when the errors are dependent and heterogeneous since no estimates for the variance-covariance matrix of the responses is needed, in order to adjust for the errors. BSIMEX's estimator was found to be approximately unbiased and more efficient than the estimators found using the regression calibration method.

The classification of the performance of the different adjustment approaches with difference error structures is not surprising. In the literature of EIV, this is a common practice, as discussed in Chapter 2. Therefore, careful considerations should be taken when adjusting for the EIV for the structure of these errors and how they occur in the design points.

5.2 Future Work

During the research we found some problems that require future work. These problems take into account either the study of the EIV combined with another serious concern in fitting regression models or new practical scenarios.

In Chapter 3, we studied the effect of mixing errors using unconstrained design regions. In practice this is not commonly the case and it is possible to impose certain limits on the maximum and minimum proportion a component could take in a mixture which lead constrained or irregular design regions. In future work, the effect of mixing errors could also be examined using irregular design regions and also different models.

In Chapter 4, we only examined empirically the effect of non-Gaussian errors on the estimates of logic50. We found that the nonlinear least squares approach produces biased and inefficient estimates. However, we do not address the analytical formulas for the bias in the estimates. Therefore, further work is needed to develop such formulas and show the relation between the bias in the estimates and other model parameters, which may help to provide alternative correction approaches.

The problem in Chapter 4 was investigated using an example from bioassay dose response study. The 4-paramter logistic model was used to illustrate the results. However, other examples can be used to demonstrate the problem of study. For example, nonlinear response models are commonly used in pharmacokinetic (PK) studies. These studies can be defined as 'the study of absorption, distribution, metabolism, and elimination of drugs' (Rescigno, 1997), or simply as a study of compounds over a period of time. Such type of studies is mandatory to develop any drug.

Few people examined the problem of EIV in PK studies, for example, Wang and Dividian (1996), Jia and Nedelman (1996), D'Argenio (1981), Sun et al. (1996) and Sheiner and Beal (1981). Different reasons for the gap of investigating measurement error problems in

this area were provided by Tod et al. (2002), such as, the error in predictors are assumed to be neglected and cannot have a serious effect of the estimates of parameters. Another major reason is the lack of comparisons between the different correction methods in pharmacokinetics studies, whereas most of the comparisons are made in the epidemiological studies. Therefore, the area needs more investigation.

Wang and Dividian (1996) studied the effect of the error in the time recoded of blood samples on the analysis of a pharmacokinetics study. Jia and Nedelman (1996) also investigated the problem of measurement error in the time variable using a simulation approach and concluded that measurement error cause the analysis to be biased. Other tend to use an optimality criteria (e.g. optimal sampling times), to reduce the effect of errors on the recorded times. Such criteria proved that the estimates of parameters are more efficient than those obtained without considering the error in times (D'Argenio, 1981). Sun et al. (1996) examined the effect of random and systematic errors in sampling times in random and fixed effect models recommending correction actions to reduce the effect of such errors on parameter estimates in different designs. Sheiner and Beal (1981) suggested the use of specific means (e.g. the amount of bias, mean prediction error) to assess the difference between the observed and unobserved variables in the study and the effect of those differences on the parameter estimates. However, the disadvantage related to that is the failure to apply the assessment means if the unobserved true predictor cannot be known, such as, in observational studies.

To expand the usefulness of the proposed correction approaches, PK models can be examined with the assumption of non-Gaussian errors in the time variable since it is possible in many PK studies. Such studies could contain either classical or Berkson errors. For example, in a study to administer a new drug, a group of patients is usually assigned to take certain doses of the drug at fixed time points. At the end of the study, the experimenter could

ask the patients to report the time in which the drug was taken. Hence if the self reported times have been used as the independent variable, any errors in the time reported are then classified as classical errors. However, if the experimenter used the time variable designed for the study, the errors in the time variable can be then classified as of Berkson type. Because of these uncertainties, the results from such studies have been found to be informative and not accurate. As a solution, practitioners applied the idea of sampling window designs. In these designs, the experimenter specifies an interval of time in which the measurements should be taken instead of fixed time points (Bogacka et al., 2008). The actual times when the measurements were taken may never be known. It would be interesting to study the effect of the errors and develop an adjustment approach of analysis.

The errors in the response variables can be either homogeneous or heterogeneous. In this work we have chosen the response error to be homogeneous and with small size in order to focus on the effect of EIV on the analysis of response models. This is not the common case in practice. So, known estimation problems when σ_ε^2 is not fixed were not addressed here, hence it is worth to study the effect of EIV on the parameter estimates when the response is heterogeneous.

Appendices

Appendix A

Delta Method

The delta method (Meyer, 1965, p.128) helps in obtaining the statistical properties for some functions of one (or more) random variable(s). It is a well known approximation tool for the mean and the variance of these functions. Suppose now that $X_1,\ldots,X_n$ are independently distributed random variables with $E(X_i)=\mu_i$, $V(X_i)=\sigma_i^2$, $i=1,\ldots,n$. Let Z be a function in $X_1,\ldots,X_n$ given by $Z=H(X_1,\ldots,X_n)$. In addition assume the function $H(X_1,\ldots,X_n)$ has nonzero first and second order derivative with respect to $X_1,\ldots,X_n$. A second-order Taylor series expansion of the function $H(X_1,\ldots,X_n)$ is given by

$$H(X_1,\ldots,X_n)\approx H(\mu_1,\ldots,\mu_n)+\sum_{i=1}^{n}(X_i-\mu_i)\left.\frac{\partial H}{\partial X_i}\right|_{X_1=\mu_1,\ldots,X_2=\mu_2}$$
$$+\frac{1}{2}\sum_{i=1}^{n}(X_i-\mu_i)^2\left.\frac{\partial^2 H}{\partial X_i^2}\right|_{X_1=\mu_1,\ldots,X_2=\mu_2} \qquad \text{(A.1)}$$

Now assume that $E(X_i)=0$, for $i=1,\ldots,n$, taking the expectation of both sides of equation (A.1), gives the second-order approximation for the mean of Z

$$E(Z) \approx H(\mu_1, \ldots, \mu_n) + \frac{1}{2}\sum_{i=1}^{n} \sigma_i^2 \left. \frac{\partial^2 H}{\partial X_i^2} \right|_{X_1=\mu_1,\ldots,X_2=\mu_2} .$$

As regards to the variance, for simplicity only the first-order approximation is used here. It is given by

$$V(Z) \approx \sum_{i=1}^{n} \left(\frac{\partial H}{\partial X_i} \right)^2_{X_1=\mu_1,\ldots,X_2=\mu_2} .$$

Appendix B

Gamma Distribution

If a random variable X follows a Gamma distribution with shape γ and scale δ parameters, the density function of X can be written by

$$f(X = x; \gamma, \delta) = x^{\gamma-1} \frac{e^{-x/\delta}}{\delta^{\gamma}\Gamma(\gamma)}, \quad \text{for } x \geq 0,\ \theta, \lambda \geq 0 .$$

The mean and variance of X are $\gamma\delta$ and $\gamma\delta^2$, respectively.

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