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A Bayesian Approach to Dose-Response Assessment and Synergy and Its Application to In Vitro Dose-Response Studies

Violeta G. Hennessey,
Department of Biostatistics, The University of Texas Graduate School of Biomedical Sciences at Houston, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030, U.S.A

Gary L. Rosner,
Department of Biostatistics, The University of Texas Graduate School of Biomedical Sciences at Houston, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030, U.S.A

Robert C. Bast Jr., and
Department of Experimental Therapeutics, Office of Translational Research, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030, U.S.A

Min-Yu Chen
Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital Linkou Medical Center, Chang Gung University, College of Medicine, Taoyuan, 333, Taiwan

Violeta G. Hennessey: v.g.hennessey@mdanderson.org; Gary L. Rosner: glrosner@mdanderson.org; Robert C. Bast: rbast@mdanderson.org; Min-Yu Chen: e12013@adm.cgmh.org.tw

Summary

In this article, we propose a Bayesian approach to dose-response assessment and the assessment of synergy between two combined agents. We consider the case of an *in vitro* ovarian cancer research study aimed at investigating the antiproliferative activities of four agents, alone and paired, in two human ovarian cancer cell lines. In this study, independent dose-response experiments were repeated three times. Each experiment included replicates at investigated dose levels including control (no drug). We have developed a Bayesian hierarchical nonlinear regression model that accounts for variability between-experiments, variability within experiments (i.e., replicates), and variability in the observed responses of the controls. We use Markov chain Monte Carlo (MCMC) to fit the model to the data and carry out posterior inference on quantites of interest (e.g., median inhibitory concentration IC_{50}). In addition, we have developed a method, based on Loewe additivity, that allows one to assess the presence of synergy with honest accounting of uncertainty. Extensive simulation studies show that our proposed approach is more reliable in declaring synergy compared to current standard analyses such as the Median-Effect Principle/Combination Index method (Chou and Talalay, 1984), that ignore important sources of variability and uncertainty.

Keywords

Combination Index method; Drug interaction; Emax model; Interaction index; Median-effect principle; Loewe additivity model

Supplementary Materials
Web Table 1, Web Table 2, and Web Appendix referenced in Sections 5.1 and 5.2 are available under the Paper Information link at the Biometrics website http://www.biometrics.tibs.org.

1. Introduction

In recent years, thousands of papers have been published in the field of drug discovery suggesting promise of an agent or a combination of agents for disease treatment. Dose-response assessment and drug interaction analysis play an integral part in drug discovery and are active areas of research (e.g., Chou and Talalay, 1984; Berenbaum, 1989; Greco, Park, and Rustum, 1990; Chou and Rideout, 1991; Tallarida and Raffa, 1996; Goldoni and Johansson, 2007; Lee and Kong, 2007; Boik, Newman, and Boik, 2008; among others). The most commonly used method in published analysis is the Median-Effect Principle/ Combination Index method (MEPCI) proposed by Chou and Talalay (1984). There is a great need for improving current standard analyses. Boik et al. (2007) found current standard analysis includes preprocessing of data, often leading to inefficiency by throwing away data points and inducing correlation. Boik et al. (2008) also showed through a simulation study that standard analysis with MEPCI led to confidence intervals having coverage below the nominal value. Lee and Kong (2007) suggested that confidence intervals found in published analyses are often constructed under an unmet assumption of normality.

In this article we propose a Bayesian approach to dose-response assessment and assessment of synergy between two combined agents. We focus on *in vitro* dose-response studies and limit our work to combination studies using a single-ray experimental design, that is, agents are combined using a fixed-dose ratio. The motivating example comes from an *in vitro* ovarian cancer research study aimed at investigating the antiproliferative activities of four agents, alone and paired, in human ovarian cancer cell lines SKOV-3 and HEY. The agents were DNA methylation inhibitors, decitabine (DAC) and azacitidine (AZA), and histone deacetylase inhibitors, suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA). For each agent and cell line combination, ten dose levels were investigated, including control (no drug). Independent experiments were repeated three times. Each experiment included three replicates per dose level. After five days of treatment, sulforhodamine B (SRB) assay (Skehan et al., 1990) was used to evaluate optical density measurements (OD) as a measure of the number of cells surviving.

Many *in vitro* dose-response studies include repeated experiments carried out under varying conditions. Investigators usually repeat independent experiments to adhere to the theme of reproducibility or to satisfy journal publication standards. Analyses, however, generally do not acknowledge several important sources of variation inherent in the data, such as variability between-experiments, variability within-experiments (i.e., between-replicates), and variability in the observed responses of the controls (no drug). Variability may be attributable to a number of factors, including biology, measurement error, assay precision, and varying environmental conditions. Accounting for sources of variation will improve inference when fitting dose-response curves and analyzing drug interaction.

The first step in dose-response modeling is to choose a structural model that characterizes the relationship between an agent's dose (or concentration) level and some measured response (Greco et al., 1995). In this article, we use dose and concentration interchangeably. For a continuous response, two common structural model choices are the Median-Effect model (Chou, 1976; Chou and Talalay, 1984)

$$f_a/f_u=(D/D_m)^m, \quad (1)$$

and the Emax model (Greco, 1995; Lee et al., 2007)

$$Y = \frac{E_0 \left(\frac{C}{IC_{50}}\right)^m}{1 + \left(\frac{C}{IC_{50}}\right)^m}, \quad (2)$$

derived from the Hill equation (Hill, 1910). In the Median-Effect model (1), f_a represents the fraction of cells affected at dose D and f_u represents the fraction of cells unaffected ($1 - f_a$). In the Emax model (2), Y represents the measured response at concentration C, and E_0 represents the control effect, i.e., response when $C = 0$. In both models, D_m and IC_{50} are the respective concentration required for 50% inhibition, and m, known as Hill's coefficient, is a shape parameter. Both models conform to the mass action law principle with the Median-Effect model (1) being the the simpler form for relating dose and response. We chose to work with the Emax model (2), since it allows modeling the variability in the controls through the E_0 parameter. With algebraic manipulation and setting $M = -m$ in equation 2, the Emax model becomes

$$Y = \frac{E_0}{1 + \left(\frac{C}{IC_{50}}\right)^M}. \quad (3)$$

When two or more single agents are combined, drug interaction can occur. Types of interactions are additivity, antagonism, and synergy. Synergistic agents are of great interest in drug development. Synergy is when the effect of the combination is greater than the effect predicted from an additive (reference) model. Favorable outcomes include increased therapeutic benefit over single agents alone, possible decrease in dosage while maintaining or increasing efficacy, and overcoming drug resistance (Chou, 2006).

The Loewe additivity model (Loewe and Muischnek, 1926) is widely accepted as a reference model (Berenbaum, 1989; Greco, 1995; Lee et al., 2007). Loewe additivity is motivated by considering a sham experiment in which an agent is combined with itself; by definition, an agent cannot interact with itself (Greco, 1995). The Loewe additivity model (or isobole equation) for two agents is

$$1 = \frac{d_A}{D_A} + \frac{d_B}{D_B} \quad (4)$$

and can be extended to three or more agents. The numerators, d_A and d_B, represent the respective doses of drug A and drug B in combination resulting in a specific effect. The denominators, D_A and D_B, represent the respective doses of drugs A and B that by themselves result in the same specific effect as the combination. In the case of additivity, the sum equals one. This sum, called the Loewe interaction index, is less than one in the case of synergy and greater than one if there is antagonism.

Chou and Talalay (1984) imply that the combination index is equal to the Loewe interaction index when the drugs obey the Median-Effect Principle and the effects of the drugs are mutually exclusive (i.e., they have the same modes of action). If the drugs are mutually non-exclusive (i.e., they have different modes of actions), Chou and Talalay (1984) suggest an additional term in the sum. In practice, however, the additional term is rarely used and confidence intervals for the interaction index are constructed to perform hypothesis testing of additivity. Uncertainty in the interaction index's input parameters is ignored.

Boik et al. (2008) consider dose-response studies comprising one experiment with replicate multi-well trays performed simultaneously. The nonlinear mixed-effects model of Boik et al. (2008) only considers a random tray effect on the control effect parameter E_0; IC_{50} and M are fixed effects in their model and do not vary across trays. Boik et al. (2008) rely on asymptotics to assess synergy via confidence intervals. Reliance on asymptotics may not be appropriate in a small sample (number of experiments) setting.

In this article, we provide an alternative Bayesian framework for dose-response assessment and drug interaction analysis. Within this framework, we (1) coherently manage uncertainty through probability calculus, (2) account for multiple sources of variability simultaneously, (3) incorporate *a priori* knowledge into the current analysis, and (4) provide a straightforward method to compute other quantities of interest and their uncertainty. The Bayesian framework also allows for a more exact inference, making use of all available information, which is ideal when making decisions under uncertainty.

In section 2, we present our proposed Bayesian hierarchical nonlinear regression model. In Section 3, we discuss posterior inference using the model and introduce our proposed Bayesian Effect Interaction Index method for assessing synergy between two agents. We present results of a simulation study comparing the performance of our proposed approach to that of MEPCI in Section 4. In Section 5, we apply our proposed methods to real laboratory data from the ovarian cancer cell line study. Some concluding remarks are given in Section 6.

2. Bayesian Hierarchical Nonlinear Regression Model

We propose a three-stage Bayesian hierarchical nonlinear regression model for dose-response of a single agent or two combined agents. Our proposed model accounts for variability between-experiments, variability within-experiment (between-replicates), and variability in the observed responses of the controls. Our proposed model also allows for incorporating *a priori* knowledge into the analysis. We fit a separate model to the dose-response data for each agent and the combination.

2.1 Stage 1 (intra-experiment variation)

In Stage 1, we model the variability within-experiment (between-replicates). We propose a lognormal sampling distribution, since the outcomes are nonnegative, continuous, and generally present skewed, lognormal-like residuals. Often there is an apparent relationship between mean response and variation.

Let y_{ijk} be the measured response for replicate k in experiment i at the j^{th} concentration level C_j. The data model is $log(y_{ijk}) = \mu_{ij} + \varepsilon_{ijk}$, with $\varepsilon_{ijk} \sim N(0, \sigma^2)$. Here μ_{ij} is the mean response, and ε_{ijk} is the random residual, both on the log scale. Within-experiment variation includes measurement error, natural variation in the response within a replicate, and variation between replicates. We assume an inverse-gamma prior for σ^2. On the original scale, $Var(Y_{ijk}) = \sigma^2\mu_{ij}$, implying heteroscedasticity across dose levels and across experiments. Other error functions (e.g., $\sigma^2\mu_{ij}^{\theta}$) may also be used. The expected response on the original scale, $E(Y_{ijk})$, is the Emax model (equation 3)

$$E(Y_{ijk}) = \frac{E_{0,i}}{1+\left(\frac{C_j}{IC_{50,i}}\right)^{M_i}}.$$

We allow the parameters E_0, IC_{50}, and M to vary across experiments. When fitting the model to data from a combination study with doses at a fixed ratio $\rho = dose_B/dose_A$, we treat the concentration of A as the independent variable for convenience. Inference can be in terms of agent A or agent B, however, because of ρ.

2.2 Stage 2 (inter-experiment variation)

We model the variability between experiments with a second stage in the hierarchical model.

$$\begin{aligned} E_{0,i} &\sim LN(\mu_{logE_0}, \sigma^2_{logE_0}), \\ IC_{50,i} &\sim LN(\mu_{logIC50}, \sigma^2_{logIC50}), \\ M_i &\sim LN(\mu_{logM}, \sigma^2_{logM}) \end{aligned}$$

Log-normal prior distributions ensure positive values and a realistic skewness. Note that M is positive for antiproliferative activities.

2.3 Stage 3 (hyperprior distributions)

We complete the hierarchical model by putting priors on the hyperparameters.

$$\begin{aligned} \mu_{logE_0} &\sim N(a, d), & \sigma_{logE_0} &\sim half-Cauchy(g) \\ \mu_{logIC50} &\sim N(b, e), & \sigma_{logIC50} &\sim half-Cauchy(h) \\ \mu_{logM} &\sim N(c, f), & \sigma_{logM} &\sim half-Cauchy(l) \end{aligned}$$

We use Normal distributions, for the location hyperparameters and half-Cauchy distributions for the scale hyperparameters. Gelman (2006) proposed the use of half-Cauchy prior distributions for the standard deviations (σ) of second level parameters when the number of groups is small. If historical information is available from prior studies, we would incorporate it at this stage of the hierarchy (Davidian and Giltinan, 1995). Otherwise, we set a, b, and c to arbitrary values, such as 0, and set d, e, and f to reflect vague (flat) priors. Gelman (2006) recommends values for g, h, and l that reflect weakly informative priors, i.e., intentionally weaker than whatever actual prior knowledge is available.

We implement the half-Cauchy prior distribution for $\sigma_{logIC50}$ via the following reparameterization. There is a similar reparameterization for E_0 and M.

$$\begin{aligned} IC_{50,i} &\sim LN(\theta_{i,logIC50}, \sigma^2_{logIC50}) \\ \theta_{i,logIC50} &= \mu_{logIC50} + \xi_{logIC50} * \eta_{i,logIC50} \\ \xi_{logIC50} &\sim N(0, b^2), \quad \eta_{i,logIC50} \sim N(0, \tau^{-1}_{\eta_{logIC50}}) \\ \tau_{\eta_{logIC50}} &\sim Gamma(.5, .5) \\ \sigma_{logIC50} &= \frac{|\xi_{logIC50}|}{\sqrt{\tau_{\eta_{logIC50}}}} \end{aligned}$$

The overparameterization reduces dependence among the parameters in the hierarchical model and improves MCMC convergence (Gelman, 2006).

3. Bayesian Posterior Inference

The posterior distributions are not available analytically. We used MCMC via WinBUGS (Spiegelhalter, 2002) to simulate samples from posterior distributions of relevant parameters.

3.1 Dose-Response Assessment

Our proposed model considers each individual experiment as arising from a population. We display experiment-specific fitted dose-response curves using equation (3) with posterior estimates of $E_{0,i}$, $IC_{50,i}$, and M_i. We display a population mean dose-response curve using equation (3) with exponentiated posterior estimates of μ_{logE0}, $\mu_{logIC50}$, and μ_{logM}. We recommend the use of the median fitted response because of skewness.

Interest in other inhibitory concentrations (IC_x = the concentration producing x% inhibition) requires a function of the parameters from the fitted dose-response model where the outcome is $(1-Y/E_0)$*100. For example, the i-th posterior sample of IC_x at the population level is constructed by the following inverse function:

$$IC_x^{(i)} = \mu_{IC50}^{(i)} \left(\frac{x}{100-x} \right)^{1/\mu_M^{(i)}} \quad (5)$$

The superscript ($i = 1, \ldots, N$) refers to saved MCMC samples; N is the number of MCMC samples saved for posterior inference. Uncertainties in the parameters μ_{IC50} and μ_M propagate into uncertainty about IC_x. Uncertainty of μ_{E0} is not considered since x is a fraction of control.

3.2 Assessment of Synergy

Our objective is to assess the nature of interaction between agents A and B at different inhibitory levels. We propose the following Bayesian Effect Interaction Index method. The method bases inference on the posterior distribution of the Loewe interaction index at an inhibitory level of interest. Posterior samples of the Loewe interaction index at x% inhibition (II_x) are constructed by the following function, also known as the isobole equation,

$$II_x^{(i)} \frac{IC_{x,A+B}^{(i)}}{IC_{x,A}^{(i)}} + \frac{\rho * IC_{x,A+B}^{(i)}}{IC_{x,B}^{(i)}}. \quad (6)$$

In the numerators, $IC_{x,A+B}$ represents the inhibitory concentration of agent A in the combination (A + B) producing x% inhibition; ρ is the fixed-dose ratio used in the combination study. In the denominators, $IC_{x,A}$ and $IC_{x,B}$ represent respectively the inhibitory concentrations of agent A and B alone that yields x% inhibition. These concentrations are considered random, and we generate posterior samples using equation (5) with parameters from the agent-specific fitted dose-response model as described in Section 3.1.

We declare synergy by calculating the posterior probability that II_x is less than $1 - \varepsilon$, for some small positive ε. This posterior probability is calculated by

$$\gamma = Pr(II_x < 1-\varepsilon | data) \simeq \frac{1}{N}\sum_{i=1}^{N} \mathbf{I}[II_x^{(i)} < 1-\varepsilon], \quad (7)$$

where **I[·]** in equation (7) is an indicator function. We use $1 - \varepsilon$ rather than 1 to differentiate synergy from additivity in case II_x is close to 1. We conclude synergy if γ exceeds some threshold. For example, we might declare "Synergy" if $\gamma > 0.90$. The threshold 0.90 is an arbitrary but conservative value. The decision rule is to conclude synergy if II_x falls below 1 with high probability. One may consider other values, such as 0.80 or 0.70. We recommend a threshold that will ultimately minimize error and maximize correct decision making.

4. Simulation Study

We conducted a simulation study to evaluate and compare the performance of our proposed Bayesian hierarchical nonlinear regression model to standard analysis with the Median-Effect Principle. We present frequentist criteria, bias and mean squared error (MSE), to measure the performance of estimator accuracy and precision in dose-response curves. We also investigated the performance of the Bayesian Effect Interaction Index method and the Median-Effect Principle/Combination Index method (MEPCI) to declare synergy at different inhibitory levels. We focused on performance at the population level.

We generated dose-response data for three hypothetical agents: agent A, agent B, agent C, and agent A combined with agent C. For example, we generated dose-response data for agent A by first creating a "true" population mean dose response curve using equation (3) with parameter values $E_0 = 3$, $IC_{50} = 35$, and $M = 0.41$. We chose to use equation (3) instead of equation (2) so we could generate control responses (responses in the absence of drug). With the idea that experiment-specific means deviate from the population mean and replicates deviate from the experiment-specific mean, we generated a data set consisting of three experiments with triplicates. The simulated experiments included ten concentration levels with serial 2-fold changes, yielding 30 observations per experiment and 90 observations per agent-specific data set. We used a log-normal distribution for $E_{0,i}$ to constrain values to be positive. We used a bivariate log-normal distribution for $IC_{50,i}$ and M_i again to constrain positive values and to introduce plausible correlation between the two parameters. We then used a multiplicative error term to ensure positive data points and introduce heteroscedasticity. Between-experiment variation for parameters was based on plausible coefficients of variation.

The Median-Effect method models the variable fraction affected (f_a) (Chou and Talalay, 1984). This requires normalizing the observed data by the control response. Data points that fall outside the allowed (0,1) range are deleted. The Median-Effect model (1) is characterized as a linear regression model by regressing logit(f_a) on log(D). Ordinary least squares is used to estimate model parameters. We investigated three common analysis methods: complete-pooling, no-pooling, and weighted meta-analysis. Complete-pooling uses observed responses, normalized by their experiment-specific control responses, and fits the model to the pooled data. In the no-pooling analysis, observed responses are normalized by their experiment-specific control responses, and a separate model is fit to each experiment. Parameter estimates from the separate experiment-specific analysis are averaged. The weighted meta-analysis first averages replicates within each experiment and then normalizes by the experiment-specific average control responses. The process leads to one data point at each dose level for each experiment. One then computes a weighted average of parameter estimates from separate fits to each experiment's data, with each estimate weighted by the inverse of the standard error. We used a computer program, SYNERGY (Lee et al, 2006), to implement the Median-Effect method. We used the delta

method to approximate the standard errors for the weighted meta-analysis estimators $\hat{IC}_{25}$, $\hat{IC}_{50}$, and $\hat{IC}_{75}$.

Table 2 summarizes the results of 1000 simulations. For all all four hypothetical studies, the $\hat{IC}_{50}$ from the Bayesian model outperformed the other analyses in terms of bias and MSE. The MSE for all four $\hat{M}$ are quite small, however, our Bayesian method outperformed analysis with the Median-Effect method when estimating other inhibitory concentrations of interest, $\hat{IC}_{25}$ and $\hat{IC}_{75}$. We found performance to depend on between-experiment variation, inhibitory level, and if the parameter estimate lay in a flat region of the dose-response curve or required extrapolating beyond observed dose levels.

We also investigated the performance of our Bayesian Effect Interaction Index method and compared it to the MEPCI for three drug interaction scenarios. In Scenario 1, agent C combined with itself (sham experiment) produces no interaction. In this case, the Loewe interaction index equals 1 and additivity should be concluded at all inhibitory levels. In Scenario 2, agents A and C are combined, producing “strong” or “very strong synergy” (Chou, 2006) at all inhibitory levels. In Scenario 3, agent B combined with agent C, leads to qualitatively different interactions, that is, very strong synergy at 5% inhibition to very strong antagonism at 95% inhibition (Chou, 2006).

Our Bayesian method uses the posterior distribution of the Loewe interaction index for deciding whether synergy, additivity, or antagonism exist. In the simulations, decision making rested on the posterior probability of the Loewe interaction index being less than .95 for synergy and greater than 1.05 for antagonism. We concluded synergy or antagonism if either probability exceeded some threshold (i.e, 0.90, 0.80, or 0.70). Additivity was the absence of synergy or antagonism.

The MEPCI computes a 95% confidence interval for the interaction index and bases decisions on this interval. The SYNERGY program provides a point estimate for the interaction index and its 95% confidence interval using the delta method (Lee et al., 2007). It is suggested that one concludes synergy when the interaction index is less than one and the 95% confidence interval falls below one. One concludes antagonism, on the other hand, if the interaction index is greater than one and its 95% confidence interval lies above one. Additivity is when the 95% confidence interval includes one.

For these simulations, we used the weighted meta-analysis, which appears to be popular. Figure 1 shows, for the three drug interaction scenarios, the percentage (out of 1000 simulation realizations) of correct declarations for the MEPCI and the Bayesian Effect Interaction Index method using different thresholds (γ). We found the Bayesian method to be more reliable in this simulation study.

In Scenario 1, a sham experiment, the MEPCI led to a high fraction of decisions being false positive, incorrectly concluding no additivity across all inhibitory levels. In scenario 3, strong synergy across all inhibitory levels, the MEPCI and the Bayesian method with $\gamma >$ 0.80 and $\gamma > 0.70$ had high rates of correctly declaring synergy. The Bayesian method in this scenario performed a little worse when a large proportion of the cells were alive or dead (i.e., inhibition was close to 0% or 100%). In Scenario 4, qualitatively varying interactions, the MEPCI exhibited unexpectedly low power for most of the inhibition levels. The Bayesian approach, using $\gamma > 0.80$ or $\gamma > 0.70$, correctly declared synergy most of the time. The exception was over the narrow range when the interaction changed from synergy to additivity to antagonism (i.e., a quick change in the II_x values). In an independent simulation, however, we found that increasing the number of experiments to five or eight improved the Bayesian Effect Interaction Index method’s performance over a larger range of

inhibitory levels. Overall, we found that the Bayesian Effect Interaction Index method with $\gamma > 0.80$ maintained good operating characteristics.

5. Application to the Ovarian Cancer Cell Lines Study

We applied our model to the data from the *in vitro* ovarian cancer study. We present the analysis of the HEY cell line exposed to ten increasing concentrations (serial 2-fold changes) of DAC alone (0 to 100 μM), SAHA alone (0 to 32 μM), and DAC and SAHA together at a fixed ratio of concentrations $\rho = 0.055$. The value 0.055 arose from the drugs' relative potency, defined as the ratio of each agent's estimated IC_{50} in a prior study. When combined, DAC concentrations ranged from 0 to 262.40 μM, and SAHA concentrations were 0.055 times the DAC concentrations.

5.1 Dose-Response Assessment

We fit separate models for each agent's dose-response. For example, when constructing the model for SAHA alone, we set $a = 0$, $c = 0$, $d = 100$, $f = 100$, $g = 5$, $h = 5$, and $l = 5$ in the hyperpriors. These values reflected vague priors for the location hyperparameters and weakly informative priors for the scale hyperparameters. We set $b = 0$ and $e = 10$ by using a priori knowledge on SAHA alone; the investigators had used published literature to select the range of doses that most likely contained the IC_{50}. Since the literature they referenced included experiments with other cell lines, we let the investigated range of concentrations represent the standard deviation, leaving probability to larger uninvestigated dose levels.

We coded the agent-specific Bayesian hierarchical nonlinear regression model in WinBUGS (See Web Appendix). We ran 30,000 MCMC iterations with three chains, each with different initial values, retaining every tenth sample. We discarded the first 20,000 iterations as burn-in. We assessed convergence using standard diagnostic tools, that is, converging and mixing of the three chains. We also compared histograms of the posterior samples with their respective priors to ensure that the priors had not constrained posterior inferences.

Figure 2 shows the fitted dose-response curves for DAC alone, SAHA alone, and DAC combined with SAHA. The dashed lines represent the experiment-specific fitted curves (normalized with respect to their fitted control effect). The population mean model is shown by the solid line. DAC alone did not fit well over the entire range of investigated dose levels, owing to inherent noise and the limited activity over the study's dose range. Web Table 1 lists posterior medians and 95% credible intervals for IC_{25}, IC_{50}, and IC_{75} at the experiment level and at the population level. We note that interval estimates tended to be wide at the population level, because of the large uncertainty with only three experiments. Increasing the number of experiments would narrow our uncertainty at the population level; otherwise, we recommend the use of the median, a more robust estimator for the parameters.

5.2 Assessment of Synergy

Figure 3(a) shows population posterior predicted dose-effect curves for DAC alone, SAHA alone, and both agents combined. Visually, there appears to be synergy. Figure 3(b) shows the posterior distributions of the Loewe interaction index (II_x) as boxplots by level of inhibition (x %). Asterisks indicate inhibitory levels where II_x fell below 0.95 with high probability. Based on results of the simulation studies, we chose to use $\gamma > 0.80$ to conclude synergy. For this example, we concluded synergy for inhibitory levels 15% to 85%. Web Table 2 contains summary statistics of our assessment of synergy over a grid of inhibitory levels.

6. Discussion

It is important to determine the nature of any *in vitro* interaction correctly because of the potential risk of toxicity in animals and human subjects when drugs are combined. In Sections 2 and 3, we proposed new methodology for dose-response assessment and drug interaction analysis. Our proposed method is advantageous, because it accounts for various sources of variability and uncertainty and allows one to incorporate *a priori* knowledge into the analysis, enabling more efficient inference. We found that accounting for sources of variability and uncertainty is important to the success of dose-response assessment and drug interaction analysis. Current standard analyses using MEPCI ignore this important component of the data. Rouder and Lu (2005) suggested that unmodeled variability can lead to biased estimation, which in turn would lead to problematic inference. This is in agreement with our simulation study results that showed that dose-response assessment can be distorted when variation is ignored.

We proposed a Bayesian hierarchical nonlinear regression model that accounts for variability between-experiments, variability within-experiments, variability in the responses of the controls, heteroscedasticity, and *a priori* knowledge. We address the use of prior knowledge, since information about a single-agent's IC_{50} can often be found (e.g., in previous studies, literature). We did not jointly model the agents alone and in combination via a dose-response surface, although one can do this if one believes strongly in borrowing strength across agents.

In addition, we introduced the Bayesian Effect Interaction Index method, based on Loewe additivity, that allows one to assess the presence of synergy quantitatively. This method accounts for uncertainty in the input parameters of the isobole equation (i.e., doses of drugs, alone and combined, that produce a given effect). Unlike the MEPCI's p-value, our method provides a probability of synergy being true given the data. Our simulation studies showed that our proposal is more reliable when analyzing drug-drug interactions, compared to standard analyses with the MEPCI. Current analyses use point estimates for the input parameters and construct 95% confidence intervals using an often unmet assumption of normality. Lee and Kong (2007) suggested constructing confidence intervals for the log-transformed interaction index using the delta method to improve inference. These intervals did not prevent the MEPCI from having a high false-positive rate under an additive drug combination scenario (sham experiment) and low power when the interaction changed qualitatively.

The Bayesian Effect Interaction Index method with threshold $\gamma > 0.80$ displayed good operating characteristics under an additive drug combination scenario (sham experiment) and under a strong synergy drug interaction scenario. Interactions that varied qualitatively over a short range of doses tended to be a larger problem for our method. We have found that increasing the number of experiments from three to five (or eight) improved performances over a larger range of inhibitory levels. With cost and time being considered, we recommend increasing the number of experiments if it would improve prediction in *in vivo* or clinical studies. By repeating the *in vitro* experiments, one will achieve more precise inference at the population level.

The choice of γ and ε will be context specific. We used a simulation study to conclude which γ value to use. We set $\varepsilon = 0.05$ and $\varepsilon = 0.10$ without affecting the results. The choice of ε could affect results if the nature of interaction is "nearly additive" or "slightly synergistic" (Chou, 2006). Such interactions may not be of interest to investigators. The "nearly additive" interaction, however, may be meaningful to drug developers in a particular disease, especially if there is little toxicity.

Boik et al. (2008) proposed a related frequentist approach with a nonlinear mixed-effects model. Our model differs from Boik et al. (2008) in several ways. They consider dose-response studies consisting of one experiment with replicate multi-well trays performed simultaneously. The mixed-effects model only considers random tray effects on the control effect parameter E_0; IC_{50} and M are fixed effects that do not vary across trays. Our motivating example required us to consider three independent experiments. The parameters E_0, IC_{50}, and M are subject to between-experiment variation, which we model. In addition, we have developed a Bayesian method based on Loewe additivity to assess the presence of synergy with honest accounting of uncertainty. The approach proposed by Boik et al. (2008) relies on asymptotics for inference. Inference with our model does not rely on asymptotics and may be more appropriate in a small sample (number of experiments) setting.

While we presented our method in the context of drug development, it has broader application. Toxicologic risk assessments require assembling data from *in vitro*, *in vivo*, and epidemiologic studies to inform the decision to call a chemical entity a carcinogen or another form of toxin. Concern over interacting chemicals, such as soldiers exposed to various prophylactic medications and pesticides during the Gulf War and interactions with the class of widely-present chemicals called phthalate esters have led to large scale studies (Petersdorf et al., 1996; Committee on the Health Risks of Phthalates, 2008) and greater awareness of potentially harmful interactions. Our method could find use in a meta-analyses of various *in vitro* and *in vivo* studies to help inform such risk assessments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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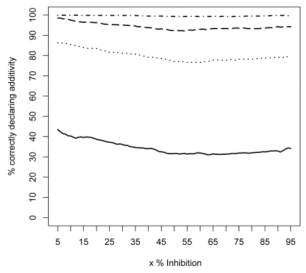
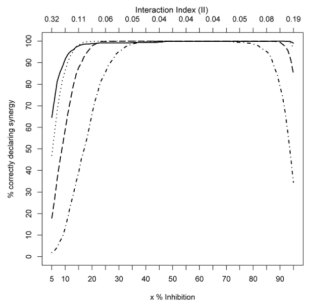
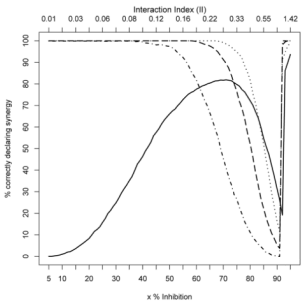


(a) Scenario 1 (sham experiment)



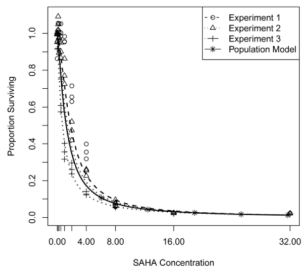
(b) Scenario 2 (very strong synergism)



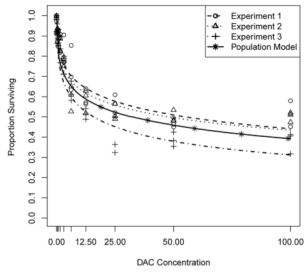
(c) Scenario 3 (qualitatively changing)

Figure 1.

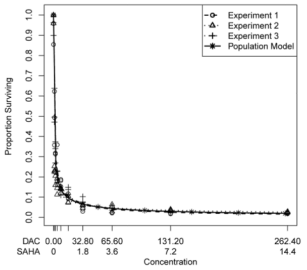
Simulation results of correct declaration for three drug interaction scenarios when three experiments are performed for each single-agent and combined-agents dose-response studies. Solid line: Median-Effect Principle/Combination Index method (MEPCI). Dash-dot line: Bayesian Effect Interaction Index method (BEII) using $\gamma > 0.90$. Dashed line: BEII using $\gamma > 0.80$. Dot line: BEII using $\gamma > 0.70$. Correct declarations: Additivity (Figures 1a), Synergy (Figure 1b), Synergy or No Synergy (Figure 1c).



(a) SAHA alone

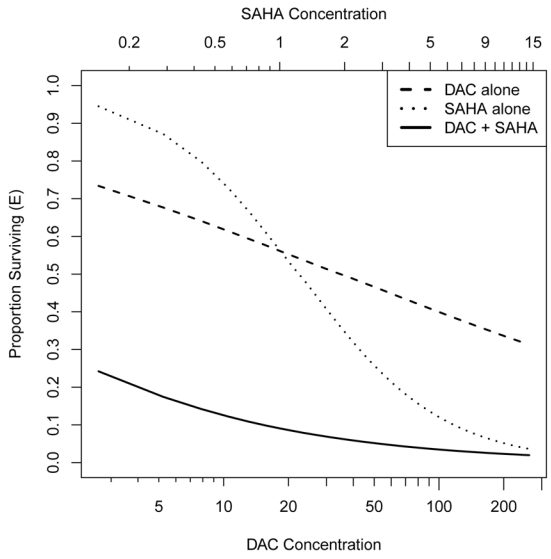


(b) DAC alone

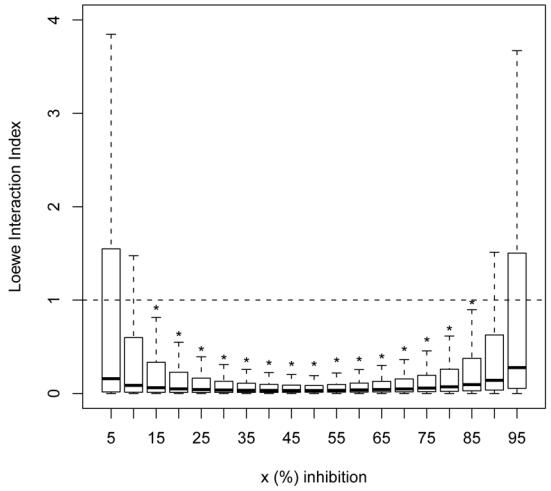


(c) DAC + SAHA

Figure 2.
Fitted Dose-Effect Curves. Experiment-specific observations are normalized with respect to their observed maximal control response. The fitted dose-response curves are normalized with respect to their fitted control effect. The solid lines represent the population mean model.



(a)



(b)

Figure 3.

(a) Population models for the three studies. Concentrations are shown on the log scale with labels on the original scale. SAHA concentrations are shown on the top axis and DAC concentrations are shown on the bottom axis. (b) Boxplots of the posterior distributions of Π_x versus x %. Asterisks indicate effect levels where Π_x falls below 0.95 $(1 - \varepsilon)$ with high probability ($\gamma > 0.80$).

Table 1

Simulation results based on 1000 simulation realizations. Comparison of estimator accuracy (Bias) and precision (MSE) of population level dose-response curves. True parameter value and variance (shown in parenthesis) is provided. σ^2 represents between-experiment (population) parameter variation.

		$\hat{IC}_{25}$		$\hat{IC}_{50}$		$\hat{IC}_{75}$		$\hat{M}$	
		2.4		**35(σ^2=196)**		**510**		**0.41(σ^2=0.0014)**	
agent	**Estimator**	**Bias**	**MSE**	**Bias**	**MSE**	**Bias**	**MSE**	**Bias**	**MSE**
A	BHNR	−0.23	0.73	−2.04	95.27	13.32	39550	−0.005	0.0012
	MECP	−1.18	1.82	−12.38	213.28	−42.95	43892	−0.040	0.0032
	MENP	−0.95	1.51	−10.26	180.26	74.92	109986	−0.039	0.0032
	MEMA	−0.30	1.07	−5.89	126.15	−84.88	41314	0.004	0.0021
		$\hat{IC}_{25}$		$\hat{IC}_{50}$		$\hat{IC}_{75}$		$\hat{M}$	
		4.78		10(σ^2=16)		20.9		1.49(σ^2=0.018)	
		Bias	MSE	Bias	MSE	Bias	MSE	Bias	MSE
B	BHNR	−0.35	1.02	−0.60	4.77	−0.97	23.90	−0.003	0.006
	MECP	−2.36	5.96	−3.66	16.02	−4.13	42.28	−0.33	0.126
	MENP	−2.11	5.00	−2.98	12.66	−1.85	45.98	−0.32	0.110
	MEMA	−0.98	2.16	−1.67	8.21	−2.44	34.47	−0.09	0.027
		$\hat{IC}_{25}$		$\hat{IC}_{50}$		$\hat{IC}_{75}$		$\hat{M}$	
		0.62		1.38(σ^2=0.30)		3.08		1.37(σ^2=0.015)	
		Bias	MSE	Bias	MSE	Bias	MSE	Bias	MSE
C	BHNR	−0.04	0.019	−0.07	0.10	−0.11	0.59	−0.004	0.005
	MECP	−0.21	0.055	−0.39	0.21	0.63	0.85	−0.140	0.027
	MENP	−0.18	0.047	−0.31	0.17	−0.40	0.75	−0.127	0.025
	MEMA	−0.09	0.031	−0.19	0.15	−0.36	0.81	−0.0157	0.010
		$\hat{IC}_{25}$		$\hat{IC}_{50}$		$\hat{IC}_{75}$		$\hat{M}$	
		0.13		0.69(σ^2=0.07)		3.56		0.67(σ^2=0.0036)	
		Bias	MSE	Bias	MSE	Bias	MSE	Bias	MSE

		$\hat{IC}_{25}$		$\hat{IC}_{50}$		$\hat{IC}_{75}$		$\hat{M}$	
		2.4		**35(σ^2=196)**		**510**		**0.41(σ^2=0.0014)**	
agent	**Estimator**	**Bias**	**MSE**	**Bias**	**MSE**	**Bias**	**MSE**	**Bias**	**MSE**
A + C	BHNR	−0.008	0.0010	−0.03	0.028	−0.045	1.02	−0.002	0.0014
	MECP	−0.030	0.0016	−0.14	0.039	−0.624	1.11	−0.008	0.0015
	MENP	−0.025	0.0014	−0.10	0.035	−0.3395	1.07	−0.008	0.0015
	MEMA	−0.012	0.0011	−0.06	0.031	−0.33	1.10	0.002	0.0014

BHNR: Bayesian hierarchical nonlinear regression model

MECP: Median-Effect Complete-Pooling

MENP: Median-Effect No-Pooling

MEMA: Median-Effect weighted Meta-Analysis