■ LABORATORY INVESTIGATIONS

Flexible Interaction Model for Complex Interactions of Multiple Anesthetics

Matthew Fidler, B.S., M.Stat.,* Steven E. Kern, Ph.D.†

Background: Minto *et al.* (ANESTHESIOLOGY 2000) described a mathematical approach based on response surface methods for characterizing drug–drug interactions between several intravenous anesthetic drugs. To extend this effort, the authors developed a flexible interaction model based on the general Hill dose–response relation that includes a set of parameters that can be statistically assessed for interaction significance.

Methods: This new model was developed to identify pharmacologically meaningful interaction-related parameters and address mathematical limitations in previous models. The flexible interaction model and the model of Minto *et al.* were compared in their assessment of additivity using simulated sample data sets. The flexible interaction model was also compared with the Minto model in describing drug interactions using data from several other clinical studies of propofol, opioids, and benzodiazepines from Short *et al.* (ANESTHESIOLOGY 2002) and Kern *et al.* (ANESTHESIOLOGY 2004).

Results: The flexible interaction model was able to accurately classify an additive interaction based on the classic definition proposed by Loewe, with at most an 8% difference between the two surfaces. Also, the proposed model fit the clinical interaction data as well or slightly better than that of Minto *et al.*

Conclusions: The new model can accurately classify additive and synergistic drug interactions. It also can classify antagonistic interactions with biologically rational surfaces. This has been a problem for other interaction models in the past. The statistically assessable interaction parameters provide a quantitative manner to assess the interaction significance.

◇ This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

⊕ Additional material related to this article can be found on the ANESTHESIOLOGY Web site. Go to http://www.anesthesiology. org, click on Enhancements Index, and then scroll down to find the appropriate article and link. Supplementary material can also be accessed on the Web by clicking on the "ArticlePlus" link either in the Table of Contents or at the top of the Abstract or HTML version of the article.

---

* Graduate Student, Department of Pharmaceutics and Pharmaceutical Chemistry, † Assistant Professor, Department of Pharmaceutics and Pharmaceutical Chemistry and Department of Anesthesiology, University of Utah.

Address correspondence to Dr. Kern: University of Utah, 421 Wakara Way, No. 318, Salt Lake City, Utah 84108. steven.kern@hsc.utah.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology. org.

ANALGESICS and sedatives/hypnotics are combined to produce anesthesia through drug interactions. This combination of agents enables the anesthesiologist to create an appropriate level of anesthesia with a fraction of the amount of drug that would be necessary if either agent were used alone. Many clinical therapies for cancer, infections, and asthma, among others, intentionally use drug combinations to produce clinically significant interactions.[1–10] To optimize dosing strategies for drug combinations, various quantitative approaches have been developed. These methods fit into four general categories: isoboles, ray design, response surfaces, and purely descriptive methods.[11–19]

Minto *et al.*[15] developed a new method for modeling interactions that combined the isobole, ray design, and descriptive response surfaces. Differences in drug interactions were fit to polynomial functions to describe the Hill-based pharmacodynamic parameters for the interacting agents. This approach was an extension of previous models, such as that proposed by Greco *et al.*, by describing the interaction through factors unrelated to potency.[10,20] The focus of their method was to capture the underlying shape of the surfaces that emerged from the clinical pharmacology data rather than to produce a particular mathematical structure to represent interaction models in general.

In a previous report from our group, we developed interaction models for propofol and remifentanil for a series of experiments in human volunteers using the approach described by Greco. Although this approach has less flexibility to map the response surface, it does provide a unitary interaction parameter that allows comparison of the two-agent interaction over a range of surrogate clinical effects. From this, we noted that the relative level of interaction between these two agents was less for sedation (indicated by Observer Assessment of Alertness/Sedation score) than for blunting the response to laryngoscopy. Although the Minto model created surfaces that fit a variety of interaction data well, comparison of the degree of interaction was focused on visual qualitative methods rather than any quantitative indicator.[15]

To retain the feature of an interaction parameter for quantitative analysis, we developed a method referred to as the flexible interaction (FI) approach, where each interaction model parameter is linked to a specific phar-

286

macodynamic interpretation. The summary parameter can provide an immediate idea of the interaction curve shape and properties so that in addition to assessing the interaction surface shape, the clinician can determine with some degree of statistical confidence the interaction type, the maximum interaction point, and the interaction curve shape. We assessed the accuracy of this model using the data from Short et al.[21] that served as the basis for the development of the Minto interaction model. We also compared this new model with the Minto interaction model in its ability to meet recommended standards for defining interaction conditions and to assess its corresponding advantages and disadvantages.[9,10,15]

Materials and Methods

The FI model was developed considering traditional pharmacologic definitions of drug interactions. After its development, its capability to identify a known additive interaction surface was assessed as an indicator of its ability to accurately classify drug interactions. Classification refers to whether the model can accurately determine the type of interaction that is present from combining multiple drugs. This was compared with the model of Minto et al.,[15] which has been widely used in the anesthesia literature to classify anesthetic drug interactions. After this comparison, the FI model was used to classify the interactions of the original data set from Short et al.,[22] which served as the basis for the development of the Minto et al.[15] model. In addition, the FI model was fit to the data set of Kern et al., which allowed assessment of interactions that were asymmetric and with profound synergism.[10,20] Comparisons between the FI model fit and the Minto et al. model are made both qualitatively and statistically.[15]

Model Development

Both Loewe and Berenbaum developed a condition for noninteracting drugs, called an additive interaction.[9,10] For two drugs acting in a sigmoid pharmacodynamics manner as described by the Hill equation, this condition becomes

$$1 = \frac{[A]}{\left(\frac{E - E_0}{E_{A,max} - E}\right)^{\frac{1}{\gamma_A}} A_{50}} + \frac{[B]}{\left(\frac{E - E_0}{E_{B,max} - E}\right)^{\frac{1}{\gamma_B}} B_{50}} \quad . \quad (1)$$

Here, $[X]$, $E_{X,max}$, γ_X, and X_{50} represent the concentration, maximum effect, slope of the Hill function, and concentration where 50% of each drug's effect occurs when acting alone, respectively. E_0 represents the baseline effect when no drug is present.

The denominators of equation 1 represent the concentration of drugs A and B that give effect E when acting alone. These concentrations are often called the isoeffective concentrations. In an additive combination, the two drugs that are administered cause the same effect as the potency equivalent amount of a single drug administered alone. When plotting the concentration of one drug against another for a given level of effect, drug combinations that produce a line that intersects the isoeffective concentrations of each individual agent at the axes define additivity mathematically.

By plotting the combinations producing a set effect, isobolographs easily visualize the interactions. An alternative method visualizes the complete range of effects produced by the combination. This creates a response surface that classifies the entire range of effects by a three-dimensional plot. Additivity for a response surface may be harder to visualize than with an isobole. An additive response surface assumes that there is a linear relation between all effects. Each set effect defines an isobole in a response surface, as graphically illustrated in Minto et al.[15]

While equation 1 defines additivity for drug combinations, deviations from it can be used to classify an interaction as either synergistic or antagonistic. When an interaction is synergistic, it takes less drug in combination to produce the same effect than if only one drug is used. Graphically, the combinations of drugs producing an effect are closer to the origin/effect axis than the line/surface of additivity, and the left-hand side of equation 1 is less than 1. Conversely, when an interaction is antagonistic, it takes more drugs in combination to produce the same effect. Interaction concentrations that produce an antagonistic effect are farther from the origin/effect axis than the line/surface of additivity, and the left-hand side of equation 1 is greater than 1.

Minto et al.[15] proposed a model that can be written as follows:

$$U_{50}(\theta_p) = \frac{1}{\left(\frac{E - E_0}{E_{max}(\theta_p) - E}\right)^{\frac{1}{\gamma(\theta_p)}}} \left[\frac{[A]}{A_{50}} + \frac{[B]}{B_{50}}\right],$$

$$\theta_p = \frac{[A]/A_{50}}{[B]/B_{50} + [A]/A_{50}} \quad .$$

U_{50}, E_{max}, and γ were defined by polynomial functions for simplicity in their approach. U_{50} represents the number of normalized potency units associated with 50% of maximum effect. All other terms have the same meaning as in the standard Hill dose–effect relation. The polynomial terms are abbreviated by u_i, g_i, and z_i for U_{50}, E_{max}, and γ, respectively, where i represents the order of the polynomial by which the number is multiplied (u_2 is the constant in front of θ^2). If the polynomial is a quadratic function, the interaction can be statistically tested based on the polynomial parameter values alone. For higher order polynomials, the parameters alone cannot be sta-

tistically assessed to determine the interaction type. Hence, the ability to fit data (goodness of fit) or the strength of the interaction statement (statistical power) is sacrificed when classifying interactions that can fit higher order polynomials with the Minto model. For this reason, we were motivated to make a model that has statistically testable parameters for flexible interactions.

The FI model assumes each drug behaves in a Hill manner like the Minto model, allowing classification of interactions between drugs that have different maximum effects and different Hill slopes. When the maximum effects for the two drugs are different, the surface can still assess the interaction impact through changes in the 50% effect (EC_{50}), which is a composite indicator of the combination potency. For example, in the case of a partial agonist where one drug has a lower maximum effect than another, decreases in EC_{50} indicate a beneficial interaction, requiring less of the drugs in combination to produce the same effect as either drug alone.

Another benefit of using the Hill model is that changes in Hill slope can be predicted. Traditionally, interaction modeling assumed the Hill slopes were fixed for interactions. When observing the behavior of the drugs acting alone, each drug has Hill slope values that are usually different from one another. Therefore, it is reasonable to assume that Hill slopes can also be different in interactions.

By considering these advantages, the FI model maintains Minto's assumption, while relating directly to the previous Finney model.[10] This gives a general form of

$$
1 = \frac{1}{\left(\dfrac{E - E_0}{E_{max}(\theta_p) - E}\right)^{\frac{1}{\gamma(\theta_p)}}} \left[\frac{[A]}{A_{50}} + \frac{[B]}{B_{50}} \right.
$$
$$
\left. + \alpha \cdot f \cdot \sqrt{\frac{[A][B]}{A_{50}B_{50}}} \right]. \quad (2)
$$

The α term indicates the type of interaction. Positive values show synergism; negative values show antagonism. Classically, this interaction type is based on the assumptions of fixed maximum effect, and Hill slope. When the Hill slopes are different, both Minto and FI models deviate from the classic additive interaction surface as given by Loewe. Still, by assuming a simple linear change in the Hill slope as a function of drug fraction, predicted differences between a Loewe interaction surface and the complex Minto or FI interaction models are typically less than experimental error and therefore can be considered negligible (as shown later and displayed in fig. 1).

Further, the FI model contains a square root term inside the bracket (equation 2) for the interaction term. This structure was chosen for two reasons. First, it assures each drug fraction behaves as a new Hill-based drug, as assumed by Minto et al.[15] This ensures that the
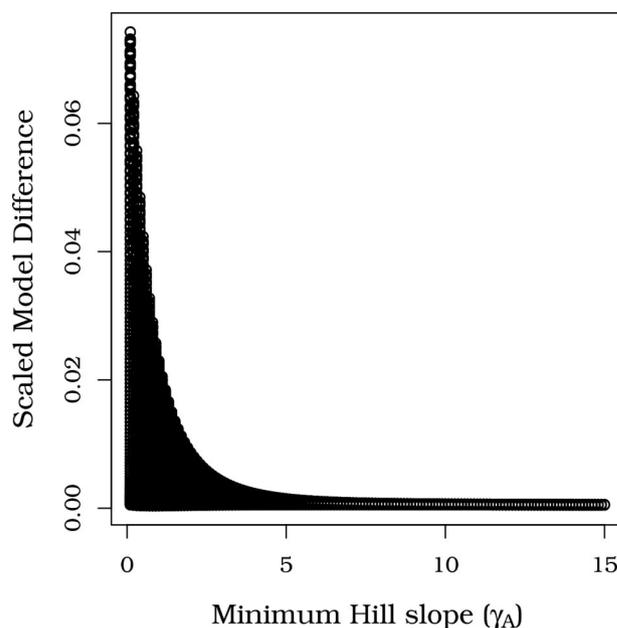

Fig. 1. The difference between either the Minto or the proposed flexible interaction (FI) model and the additive interaction surface as defined by Loewe. The FI model and the Minto model, which have the same additive interaction surface, are similar to the traditional the Loewe additive state. The scaled model difference is the sum of squared difference divided by the number of points sampled between the two "additive" surfaces.

square root allows a single solution for all isoboles, particularly those that are antagonistic. If, instead, the product alone were used, the model would have problems with antagonism. In this situation, when administering two drugs in an antagonistic combination, as in the single-constant Hill approach, the effect will increase, and then decrease with the addition of more drugs in combination. The last reason for the square root is for continuity between other models. When f = 1, and both Hill slope and maximum effect are constant, the model will be identical to the model proposed by Finney, allowing direct comparison between the two. Also, the Finney model has a mathematical relation between the interaction indexes of the single Hill constant model, as given in Greco et al.[10] Therefore, the extent of interaction can be compared directly between all three models (the extent of interaction for the FI model is equivalent to Finney's interaction index).

The disadvantage to the square root term, as discussed by Greco et al.[10] with the approach of Finney, was that it allowed the interaction surface to "fall out of the potency normalized unit square." Still, there are more benefits than drawbacks to the square root term, especially if the model is able overcome this normalized unit square problem. The key is in choosing the function f. The term f defines changes in an isobole of the response surface for a given level of drug effect. For the FI model, setting f to a functional form inspired by the γ probability distribution gives

$$f(m, w, \theta_p) = \left(\frac{\theta_p}{m}\right)^w \left(\frac{1 - \theta_p}{1 - m}\right)^w$$

$$\exp\left[-w\left(\frac{\theta_p - m}{m} + \frac{m - \theta_p}{1 - m}\right)\right]. \quad (3)$$

In this function, m is the symmetry parameter of the potency fraction, θ_p, where the maximum interaction occurs. When m = ½, the interaction is symmetric. The w parameter allows the isobole to fit to multiple shapes. If the isobole shape parameter w is set to 1, the normalized unit square problem is overcome. Similarly, the parameters γ and E_{max} are given as a function of potency fraction. Starting with a simple estimate for γ and E_{max} that is a linear function of the potency fraction, these parameters are given by the equations

$$\gamma(\theta_p) = [\gamma_A \theta_p + \gamma_B(1 - \theta_p)]$$

$$\cdot [1 + \beta f(m_\beta, w_\beta, \theta_p)]$$

$$E_{max}(\theta_p) = [E_{max,A}\theta_p + E_{max,B}(1 - \theta_p)]$$

$$\cdot [1 + \zeta f(m_\zeta, w_\zeta, \theta_p)]. \quad (4)$$

The β and ζ parameters impact the type of change in γ and E_{max}, respectively. Positive values of β and ζ indicate an increase in Hill slope/maximum effect, negative indicates a decrease, and 0 represents no change from the line. Both β and ζ are restricted to be greater than −1 to keep the respective functions positive. This condition ensures there are no negative Hill slopes or negative effects when the drug acting alone has a positive effect. The m_β and m_ζ parameters indicate the symmetry of the respective parameter change; the w_β and w_ζ parameters, which are greater than 0, represent how much a section of the curve has appreciable visibility around its location of maximum change, m_β or m_ζ. These parameters are included to allow flexibility in modeling.

Regression Procedure

Because interaction models generally have a large number of parameters, it is possible that every parameter described in the model may not be significant in determining the optimal fit. Therefore, an algorithmic step-up approach was used during regression. Selecting the best model for a given set of data can be done in three ways: (1) using *P* values to determine whether a parameter is significant, (2) a goodness of fit metric such as Akaike Information Criterion (AIC, measuring of how likely the model is given the data), and (3) using the F test based on the residuals to determine whether another parameter adds a significant descriptor to the interaction.[23] The *P* value method assumes each model is "true" when calculating the values assessing significance of parameters. The F test requires a different number of parameters to compare (which may not be true for different FI

models). Therefore, we used the AIC method of selecting a model. Smaller AIC values indicate better models. The AIC numbers have been shown in simulation to be the expected log likelihood that the data are produced by the model.[23] These numbers not only have the advantage that they are model independent, allowing two different models to be compared, but they function as a comparable measure of goodness of fit.

The ratio for the most synergistic/antagonistic drug combination in the interaction will be assumed to occur at equal potency ratios of drugs A and B. This occurs when the interaction surface is symmetric. This assumption is extended to the maximum effect and Hill slope parameter functions also. Parametrically, this is translated as m = m_β = m_ζ = 0.5, or when the potency fraction of the interacting compounds is 0.5. Unless the model-building procedure determines otherwise, a moderate value of isobole shape is assumed, w = 1. This allows the isobole/response surface to produce a gradual change to the maximum interaction. This assumption is extended to the Hill slope and maximum effect changes; thus, w = w_β = w_ζ = 1. The procedure also assumes no interaction as the null hypothesis unless the model-building procedure shows otherwise. Parametrically, this translates to α = β = ζ = 0. This approach was followed for both the FI and Minto models fit to the data.

Additive Surface Determination

The FI model and Minto model can test for interactions as defined by Loewe that are synergistic, antagonistic, and additive when E_{max} and γ are constant for all drug combinations being fit. To show this, Loewe additive surfaces (equation 1) with a constant E_{max} (θ_p) of 1 and minimum effect value of 0 were generated. The data generated were compared with the "additive" state for both the FI and Minto models, *i.e.*, constant maximum effect and the Hill slope change between the two interacting drugs modeled by a linear function.

Because the linear function between the Hill slope values for the simulated interaction surface does not force additivity everywhere (as in required in Loewe's definition) but only at the EC_{50}, differences in Hill slopes are likely to show deviations from the Loewe additive interaction surface at isoboles other than that for the 50% effect. Therefore, we tested a variety of different Hill constant combinations, which were varied from 0.1 to 15 by 0.1 increments.

We also evaluated whether changes in EC_{50} values would impact the ability of the two models to adequately classify the additive interaction surface. We simulated two surfaces where A_{50} and B_{50} were different and a surface where A_{50} and B_{50} were equal. The set of test surfaces was produced at A_{50} = B_{50} = 7.5, A_{50} = 3.75; B_{50} = 11.25, and A_{50} = 1.875; B_{50} = 13.125.

These test surfaces were created by a C++ program that generated the surface for Loewe additivity.[24] With

Table 1. Checking Model Assumptions

Drug 1	Drug 2	Minto			STP		
		AIC	Shapiro	Mean P	AIC	Shapiro	Mean P
Propofol	Midazolam	−58.96 (6)	0.3316	0.2329	**−58.97 (6)**	**0.3241**	**0.2355**
Propofol	Alfentanil	**−36.33 (6)**	**0.5739**	**0.3611**	−36.30 (6)	0.05827	0.3595
Alfentanil	Midazolam	−33.80 (5)	0.6344	0.815	**−35.09 (4)**	**0.2635**	**0.822**
All three drugs		−106.75 (10)	0.1336	0.6228	**−109.06 (9)**	**0.115**	**0.6173**

Comparison of Akaike Information Criterion (AIC) values for the Minto and proposed models. All values are model independent and assume a normal error structure. The number in parentheses represents the number of parameters used on the final model. Also compared are the P values for the Shapiro test of normality (normality is a decent assumption in all cases). The last values compared are the P values for the test ensuring the mean of the residuals is zero. This assumption is also held.

these test surfaces, the points generated were compared with both models' additive surfaces using the square difference between either model's additive surface and the Loewe generated surface. From this, we can calculate the difference between the "additive" interaction state that either model generates and the classic Loewe additive interaction state.

Validation with Data Sets

Minto *et al.* originally applied their model to a data set collected by Short *et al.*[15,22] This data set contained response data from 400 women undergoing elective gynecologic surgery who received combinations of propofol, alfentanil, and midazolam. Response to verbal command (opening eyes) was assessed at the time when the peak concentrations for the administered agents were estimated to occur (propofol and alfentanil at 2 min, and midazolam at 4 min).

The same data set was used to compare the proposed model and the Minto model using the AIC parameter. This was conducted for two-drug and three-drug interaction conditions. (Additional information regarding this is available on the ANESTHESIOLOGY Web site at http://www.anesthesiology.org.) The interaction type and Hill slope change were allowed to vary in the FI model. The Minto model was fit using a quadratic interaction relation for the potency and Hill slope values.

This comparison does not allow a full assessment of the capabilities of either model. Therefore, in addition to comparing the data from Short's study, we compared the data from the study of Kern *et al.*[20] Briefly, 12 male and 12 female subjects were studied using computer-controlled infusions of propofol and remifentanil to create an increasing staircase drug-concentration profile in each subject. The interactions were assessed with the drug alone and in combination. The following surrogate measures were used to determine effect: Observer Assessment of Alertness/Sedation score to determine sedation (1–3, not sedated; 4–5, sedated), pressure algometry on the subject's tibia, electrical tetanic stimulus at the posterior tibial nerve, and response to laryngoscopy.

These measures were assessed at various drug fractions and were then normalized to a response based on the patient and pooled. The two models were fit to this data for a more complete comparison of each model's properties.

Statistics

Unless otherwise specified, all statistical procedures and graphics were performed/generated using R.[25] When comparing two models with a different number of parameters, the F test is used as suggested in Boomer (College of Pharmacy, Oklahoma University, Norman, OK).‡

Results

The difference between the Loewe additive interaction surface and the surfaces generated by the FI or Minto model with the Hill slope parameter defined by a linear relation was minimal. Changes in A_{50} and B_{50} did not affect the differences between the additive state estimates. Changes in the parameters γ_A and γ_B caused overall squared difference between the additive surface based on Loewe and the other models that ranged from 1% to 8%. This difference is likely to be inconsequential when compared with inherent biologic variability that exists in data used to create response surfaces.

Comparing Goodness of Fit through AIC

The AIC values were essentially identical, implying that both models fit the data from Short *et al.* equally well for the interaction of midazolam or alfentanil with propofol.[21] The maximum difference in AIC was 0.02, as shown in table 1. The greatest difference in the AIC between the models for two-drug interaction models was 1.29 for the combination of alfentanil and midazolam. Although this difference is larger, it is not significantly different (P value = 0.4887). Therefore, likelihood estimates of either models are the same, indicating that both describe the data equally well. It was noted that the Hill slope decreased for the drugs in combination, which would not have been determined using the

‡ http://www.boomer.org. Accessed February 27, 2006.

Table 2. Interaction Parameter Summary for Short *et al.*[21] Data Set

	M_3	FI_3	M_{AM}	FI_{AM}	M_{AP}	FI_{AP}	M_{MP}	FI_{MP}
A_{50}	0.093 (0.003)	0.093 (0.003)	0.093 (0.003)	0.093 (0.003)	0.093 (0.003)	0.093 (0.003)	—	—
γ_A	5.276 (0.474)	5.257 (0.460)	5.643 (0.910)	5.233 (0.532)	5.686 (1.057)	5.687 (1.058)	—	—
M_{50}	0.145 (0.004)	0.145 (0.004)	0.144 (0.004)	0.145 (0.004)	—	—	0.144 (0.003)	0.144 (0.003)
γ_M	$= \gamma_A$	$= \gamma_A$	4.795 (0.748)	$= \gamma_A$	—	—	4.829 (0.567)	4.830 (0.567)
P_{50}	1.076 (0.024)	1.075	—	—	1.075 (0.029)	1.075 (0.029)	1.075 (0.020)	1.075 (0.020)
γ_P	10.633 (2.117)	11.157 (2.306)	—	—	11.167 (2.889)	11.146 (2.881)	11.162 (1.969)	11.143 (1.962)
I_{AM}	1.804 (0.082)	1.613 (0.123)	1.807 (0.095)	1.643 (0.153)	—	—	—	—
I_{AP}	0.804 (0.118)	0.491 (0.088)	—	—	0.808 (0.141)	0.507 (0.111)	—	—
I_{MP}	1.660 (0.086)	1.391 (0.116)	—	—	—	—	1.651 (0.077)	1.406 (0.109)
I_{AMP}	1.390 (0.732)	—	—	—	—	—	—	—
H_{AM}	—	—	—	—	—	—	—	—
H_{AP}	12.955 (4.404)	−0.447 (0.093)	—	—	14.103 (6.734)	−0.427 (0.142)	—	—
H_{MP}	$= H_{AP}$	$= H_{AP}$	—	—	—	—	15.153 (4.530)	−0.478 (0.093)

Parameter values and SEs (in parentheses) of the models for Short *et al.*[21] data set. Notice how invariant the parameter values are between the three-agent model and two-drug model. The subscripts denote which drugs each model uses: A for alfentanil M for midazolam, and P for propofol. The top line denotes the Minto model by M and the proposed model by STP. The Hill parameters' meanings are discussed briefly in the text. The I term represents the interaction. For the flexible interaction (FI) model, I represents the α interaction parameter; for the Minto model, the quadratic U_{50} parameter u_2. The H term represents a change in Hill slope. For the FI model, H is the β Hill slope change; for the Minto model, the quadratic change in Hill slope. Higher orders of the Minto model, and the w/m parameters of the FI model, are not significant in describing the Short *et al.* data set.

traditional parametric models described by Greco. Different Hill slopes occurred for every two-drug interaction model except the FI between alfentanil and midazolam. The parameter values are summarized in table 2.

For the three-drug interaction data, the FI model showed an improved AIC over the three-agent Minto model (*P* value < 0.001; AIC difference 2.31). The AIC measures the unbiased expected log likelihood but does not have a specific distribution. By convention, an AIC difference of 0–2 is considered weak evidence for the smaller AIC model, 2–6 is considered positive evidence, 7–10 is considered strong evidence, and greater than 10 is considered very strong evidence.[26] Therefore, the three-agent interaction model comparison of the Minto and FI models has positive evidence for the three-agent FI model. This difference is because the FI model describes the data with approximately the same variance, with one less parameter.

For the Kern *et al.*[20] data set, the FI model fits the pressure algometry, and tetanic stimuli measurements are significantly better than the corresponding Minto model (AIC difference of at least 3.24 for algometry, 13.27 for tetanic stimulus; F test; *P* value < 0.001). Both the models fit pressure algometry and tetanic stimulus better than the approach taken in the original article, which used Greco's method with a single Hill coefficient, with a minimum AIC difference of 115.00. In the case of sedation, the Minto model fit is better than the FI model, with an AIC difference of 10.08; for laryngoscopy, the FI model is better than the Minto model, with a difference of 81.49. These results are summarized in table 3.

Checking Model Assumptions

Other than goodness of fit, a model is appropriate for describing data when the error of the model is random and ideally small. Technically, the residuals are independent normal random variables with a fixed variance. This is often verified graphically and through statistical tests.

In all residual plots for Minto's two-drug model, the assumption of constant variance holds. Even at the larger "concentrations" where there are fewer data points, the data show as much variance as the points in the lower concentrations. This holds for the two-drug FI models as well. The models do not show significant deviation from the normality (as determined by the Shapiro-Wilk test), residual correlation (as determined by the Durbin-Waston test), or bias (as determined by the *t* test). The statistical tests are summarized in table 1.

The residuals for the two drug interactions are almost superimposable, validating the result that the AIC values stated—the two drug models are approximately equivalent for these data sets. The FI three-drug model and Minto three-drug model show differences in predicting the data, as shown by the AIC difference. This implies that the FI model better predicts the data than the Minto model. Examples of these fits are displayed in figures 2–5.

Practical Considerations

Both models have the same sort of descriptive properties. They produce similar-looking surfaces with different abilities to classify interactions. With the FI model, a direct transformation of the α parameter expresses the percentage increase/decrease of the 50% effect when compared with each drug in the combination acting independently. This is a unique feature of this model when compared with spline or polynomial approaches. For example, the interaction of alfentanil and midazolam requires 55% of the additive drug combination to produce the same effect at the most effective ratio; alfentanil and propofol takes 80%; and midazolam and propo-

Table 3. Comparison between SHC, Minto, and FI Model Fits to Kern *et al.*[20] Data Set

	FI Model				Minto			
	Alg.	Tet.	Sed.	Lar.	Alg.	Tet.	Sed.	Lar.
P_{50}	2.82 (0.16)	5.10 (0.24)	1.81 (0.12)	7.45 (0.38)	3.07 (0.28)	4.78 (0.22)	1.89 (0.10)	6.19 (0.40)
γ_P	1.04 (0.11)	3.57 (0.32)	4.38 (0.40)	4.00 (0.59)	1.10 (0.13)	3.20 (0.29)	5.17 (0.70)	2.51 (0.36)
R_{50}	6.25 (0.70)	20.53 (1.73)	12.25 (0.57)	67.31 (5.19)	5.48 (0.64)	18.73 (1.55)	12.24 (0.61)	55.63 (2.65)
γ_R	$= \gamma_P$	1.22 (0.142)	$= \gamma_P$	$= \gamma_P$	$= \gamma_P$	1.19 (0.14)	3.71 (0.60)	7.48 (1.75)
α/u_2	2.63 (0.68)	3.55 (0.33)	1.46 (0.20)	6.42 (0.56)	5.90 (1.93)	16.25 (4.54)	20.86 (8.03)	8.26 (4.48)
m/u_3	—	0.82 (0.05)	—	0.94 (0.03)	−2.64 (1.27)	−24.21 (6.50)	−29.75 (10.79)	−13.90 (6.37)
w/u_4	3.67 (1.26)	0.82 (0.05)	0.14 (0.20)	0.11 (0.07)	—	—	14.48 (4.72)	8.43 (2.93)
β/g_2	5.66 (1.92)	—	—	—	54.08 (8.87)	—	—	—
m_β/g_3	0.90 (0.02)	—	—	—	−40.48 (6.64)	—	—	—
w_β/g_4	—	—	—	—	—	—	—	—
AIC	**192.94**	**−77.54**	73.35	**100.75**	196.18	−63.27	**68.10**	182.24
SD	0.2859	0.2212	0.2552	0.2608	0.2873	0.2247	0.2533	0.2800
AIC_{SHC}	312.79	227.59	206.34	297.68	312.79	227.59	206.34	297.68
SD_{SHC}	0.3575	0.2796	0.2551	0.3000	0.3575	0.2796	0.2551	0.3000

Remifentanil and propofol Akaike Information Criterion (AIC) and parameter values for Kern *et al.* data set.[20] Compares the fit for pressure algometry (Alg.), tetanic stimulus (Tet.), and laryngoscopy (Lar.) for STP and Minto models. Additionally, the AIC and SD (residuals) of both models are compared, along with the single Hill constant (SHC) model proposed in the original article. The AIC_{SHC} and SD_{SHC} show the corresponding values for the original model proposed in the Kern *et al.* article. The u constants represent the U_{50} polynomial for the Minto *et al.* model; g constants, Hill slope polynomial. The subscript denotes the order of the polynomial each constant is multiplied before. All other constants' meanings are discussed in the article.

FI = flexible interaction.

fol takes 59%. These results imply the interaction is synergistic. For the Kern *et al.*[20] data set, the pressure algometry response takes 43% of the additive concentra-tion at its maximum interaction location, tetanic stimulus takes 40%, sedation takes 58%, and laryngoscopy takes 40%.

Another interesting feature is that the FI model can predict the best ratio of drug for synergistic effect. For
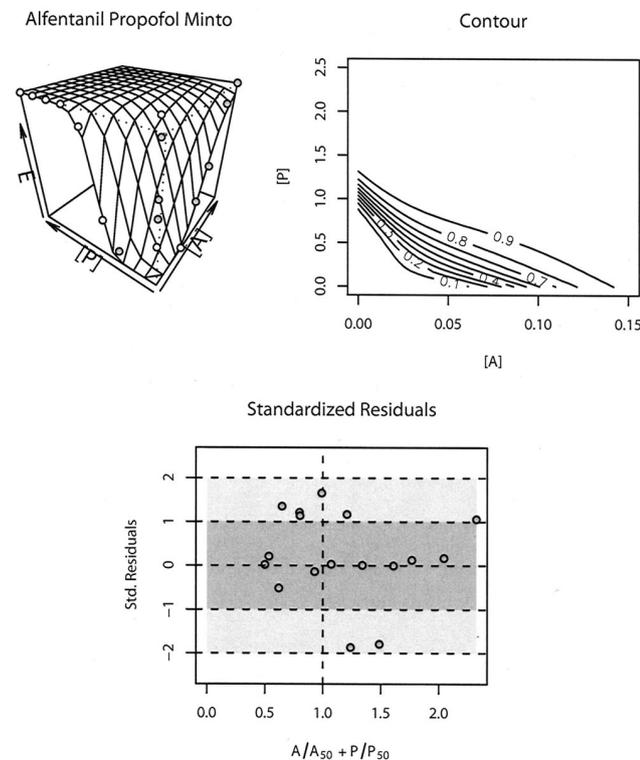

Fig. 2. The response surface fit of the interaction data from Short *et al.*[21] for alfentanil and propofol using the Minto model and residual plots. *Top left* is the response surface, with points plotted as *black triangles* if they are more than 2 SDs away from the surface, *gray filled circles* if they are more than 1 SD away, and *circles* if they are within 1 SD of the surface prediction. The *top right* shows the surface contour. The *bottom* shows the standard-ized residuals.
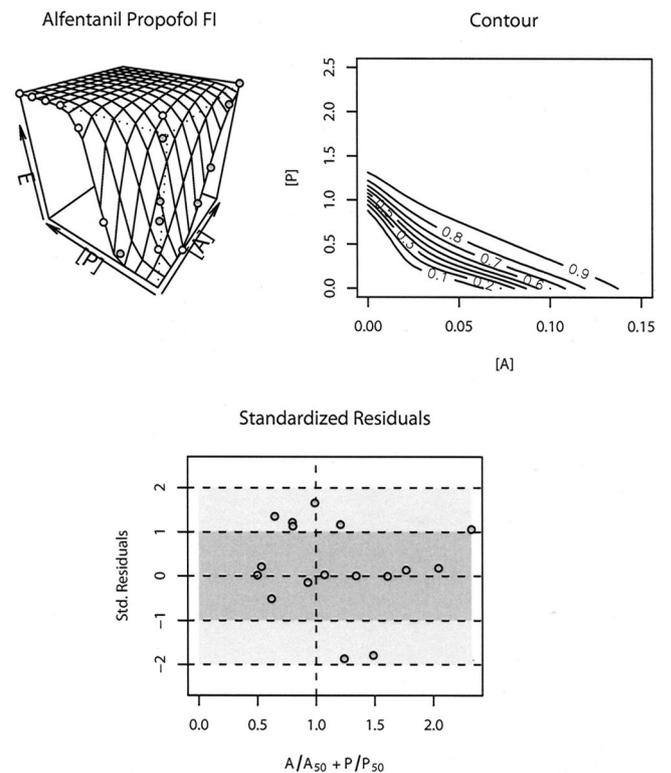

Fig. 3. The flexible interaction (FI) model fit of the interaction data from Short *et al.*[21] for alfentanil and propofol. The plots are the same as described in figure 2. For this data set, the FI model prediction is similar to that in figure 2 from the Minto data.
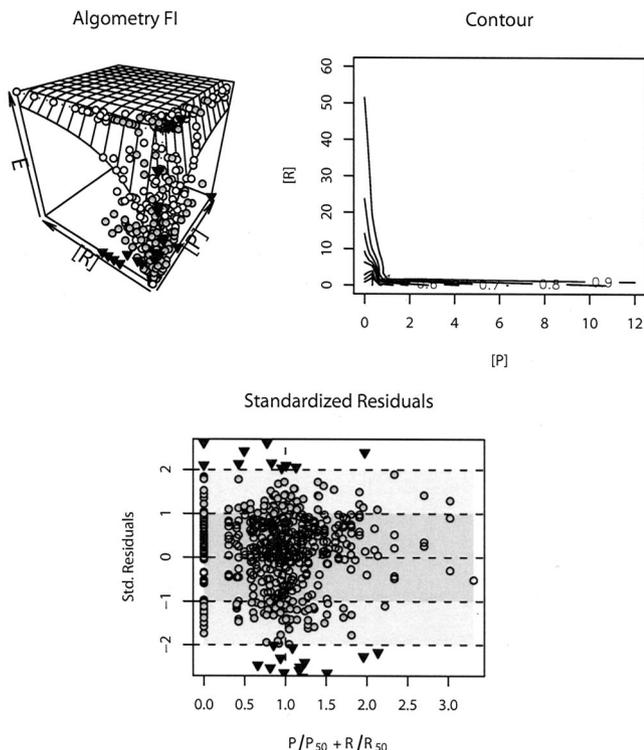
Fig. 4. The flexible interaction (FI) fit of the remifentanil and propofol interaction effect on algometry from the study of Kern et al.[20] The individual plots are the same as described in figure 2. The interaction shows significant synergism, and the model provides a good fit to the data as indicated by the surface and diagnostic plots.

fective combination. For laryngoscopy, the ratio is 1.645, implying that the combination with greater hypnotic is the best. For the other surrogate effects used in the study, the best ratio of effect of propofol to remifentanil is P_{50}/R_{50}.

Both models have an interesting property that is observable in the contours of the alfentanil and propofol interaction. The synergisms for the lower levels of the population response are greater than the higher levels of population response. Therefore, when administering propofol and alfentanil, the population that responds earlier is more sensitive to the interaction than interactions that occur later. Although this is a biologically reasonable result, it is something that could not be shown without a decrease in Hill slope. This same sort of property is exhibited to a greater or lesser degree whenever the Hill slope changes are significant and negative. When the Hill slope change is positive, the higher population is more sensitive to the interaction than the lower population. This is shown in figures 2 and 3.

Most of the surfaces seemed to generally predict the same sort of response in the Kern et al. data set except for the algometry response.[20] The Minto model predicts a decrease in Hill slope, whereas the FI does not. Both may be reasonable. The difference between the fits is shown in figures 4 and 5.

Discussion

The new FI model presented is able to fit data from the clinical experiment done by Short et al.[21] as well as the model proposed by Minto et al.[15] for two agent interac-

the asymmetric case, the ratio that gives the best pressure algometry effect of propofol to remifentanil is 0.452, i.e., a combination with the opioid at almost twice the equipotent amount as the hypnotic is the most ef-

Fig. 5. The Minto model fit of the same data from Kern et al.[20] shown in figure 4. The interaction surface difference is due to the decrease in Hill slope predicted by the model. The diagnostic plots indicate that the fit to the data is good even though the surface is irregular. This example highlights that even when the model fits the data well, the surface must be examined for suitability to describe the interaction.
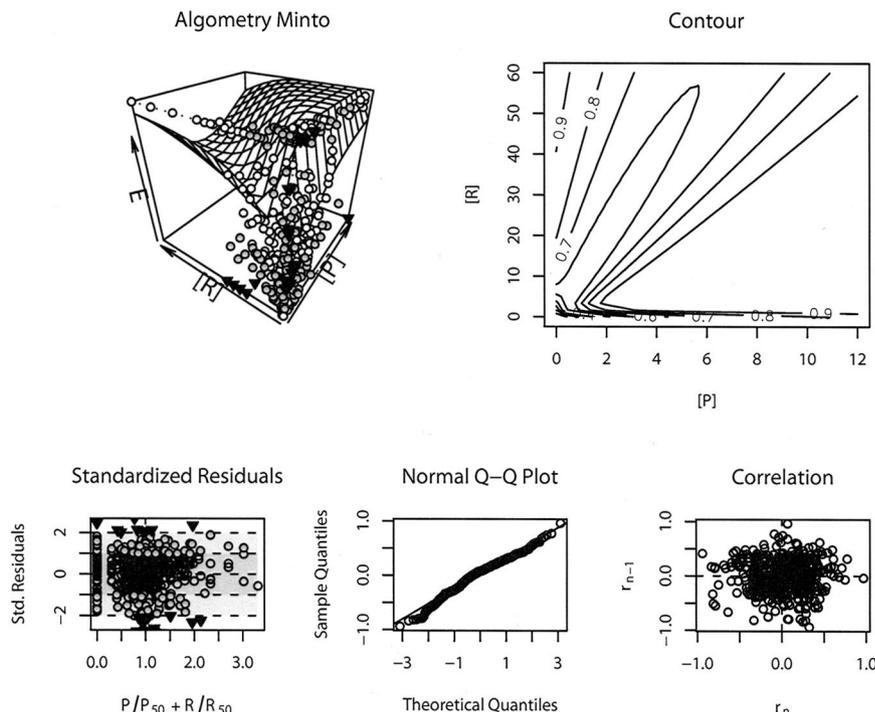
tions, and better for three interactions, while preserving the statistically testable parameter structure that allows for easy classification of the interaction type. When fitting the data set from Kern *et al.*, the fit was usually better while also preserving the parameter structure.[20] Although the necessity of this classification ability has been argued, we believe that from a clinical perspective, the classification provides the most useful reason for creating these surfaces.

Characterizing the surface description with a model describes what is happening biologically, whereas classifying what is happening with a structural model provides the ability to both adequately describing the model and test statements about the surface that are important to the understanding of interactions. The FI model goes further than many of the simple parameter-based interaction models by allowing multiple statements about the interactions to be made through testing the parameters themselves. The parameters allow assessment of whether some combination of drugs will produce a synergistic or only an additive effect. These parameters not only allow quantification the interaction type, but also allow some relative indicator of the magnitude of that interaction. In addition, the drug ratio that shows the most effective point in the interaction compared with either drug acting alone can be described by parameters.

The difference in fit between the FI and Minto models for the Kern *et al.*[20] data set could be because of the strange shape of Minto's synergistic isoboles. This isobole problem can be overcome by changing Minto's $U_{50} = 1/V_{50}$, where V_{50} is a fourth-order polynomial under the same constraints as U_{50}. Using this model gives consistently better results than the original Minto model for the Kern data set, as shown by the AIC numbers: laryngoscopy, 128.11; sedation, 65.92; algometry, 195.17; and tetanic stimulus, −74.87. Still, the FI model fits the fits the data better than the transformed Minto model, with the only exception being sedation. Therefore, part of the problem is inherent in the polynomials' descriptive power, which is discussed later.

FI Parameter Meanings

Intensity/Type Parameters. The most obvious parameters with meaning are α, β, and ζ. These determine the type and intensity of change of their respective functions as a result of the interaction. The α parameter determines the change in U_{50}/EC_{50}. Positive α values indicate decreases in U_{50}/EC_{50} from the additive state, indicating synergy. Therefore, when α is positive, less drug in combination is required than if the drugs were the same. Negative values indicate increases in U_{50}/EC_{50} from the additive state, or antagonism. The α parameter also indicates the relative amount of increase/decrease in interaction.

The β parameter represents the increase (positive) or decrease (negative) in Hill slope. Like the α parameter, there is a direct relation between the percent increase/decrease in Hill slope from the line between the Hill slopes of the drugs: $p = 1 + \beta$. Hence, a β of −0.5 represents that only 95% of the line value at the maximum Hill slope change is observed, or a decrease in Hill slope. Because a negative percent increase in Hill slope and negative Hill slopes do not make sense, β must be greater than −1. This β parameter changes the isobole shape, or degree of isobole bowing, above and below the 50% effect. This was considered to be a disadvantage of Finney's model in Greco *et al.*,[10] but is an advantage here because it allows different types of bowing to occur, not just the bowing defined by Finney's original model.

The ζ parameter represents increase/decrease of Hill slope in relation to the maximum effect line. Like the rest of the parameters, an increase is denoted by a positive value and a decrease is denoted by a negative value. The ζ parameter is also directly related to the percent increase/decrease of the maximum effect line: $p = 1 + \zeta$, where p represents the percent change from the line. Therefore, the ζ parameter represents the percent change in maximum effect in the same was as the β parameter.

Symmetry/Ratio Parameters. The symmetry/ratio parameters determine the point of maximum interaction for each drug combination. The m value indicates the potency fraction where the isobole deviation from the additive curve is the greatest, which is indicative of the most effective potency ratio for the interaction. The other symmetry parameters have similar meanings. The potency fraction where the maximum change in Hill slope and maximum effect occur is denoted by m_β and m_ζ.

The possibility of an asymmetric response surface was shown by Dahan *et al.*[27] and Niewenhuijs *et al.*[28] when modeling the interaction of alfentanil with sevoflurane and also remifentanil with propofol. Dahan's group used a spline model with a symmetry parameter to describe the data. They also used a different underlying pharmacodynamic model instead of the Hill model described in this article. For this reason, this model cannot be directly compared with their data set without modifying the underlying pharmacodynamic interaction model. Still, when modeling the data from Kern *et al.*,[20] there are changes in both symmetry and how the interaction changes from the maximum interaction drug ratio to the drug acting alone in comparison with the original model. The FI model structure handled these challenges well.

Interaction Scope Parameters. The w parameter indicates the uniformity of the interaction across various drug ratios. For a large w value, the interaction does not occur as quickly as the more uniform interaction when w = 1. For example, with a w value of 10, the interaction occurs only over the potency fraction 0.2–0.8.

Therefore, with larger values of w, a narrower region interacts. Conversely, with w values less than 1, the interaction occurs more quickly. For example, values less than ½ indicate an almost immediate interaction. These same sorts of descriptive properties are applied to the changes in Hill slope with w_β and maximum effect with w_ζ.

Novel Ways FI Model Describes Interactions

This new model has some unique properties. First, it overcomes problems that previous interaction models had with antagonistic interaction surfaces while still maintaining interaction descriptive properties through parameters. The FI model also allows interactions to be asymmetric and have a different maximally efficient drug ratio than A_{50}/B_{50} (first suggested through splines in Dahan *et al.*).[27] The last is the new interaction range described by the w parameter. This provides a description of how the interaction changes from maximum interaction to no interaction. It also allows a flexibility not achieved by the statistically testable models in the past.

With the exception of multiple interactions on the same effect slice, the FI model describes the same sorts of interactions as the Minto model: additive interactions, synergistic interactions, antagonistic interactions, partial agonistic interactions, competitive antagonist interactions, and inverse agonist interactions. In fact, the parameters can more concisely describe certain types of interactions. An example of an asymmetric interaction that is better described by fewer parameters in the FI
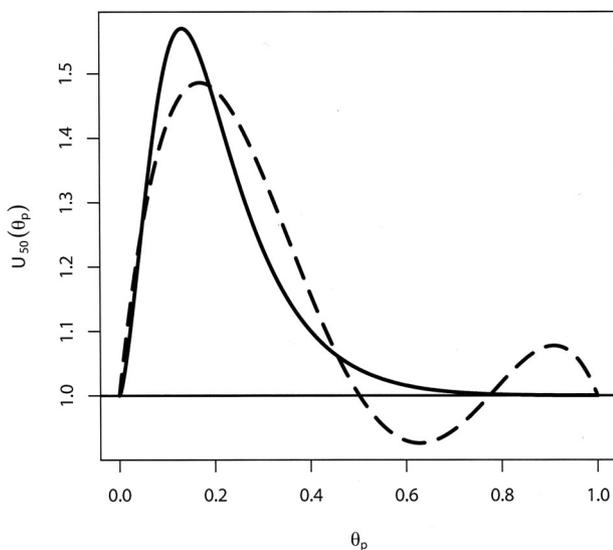
model is given in figure 6. Both the Minto and related nonconstrained spline methods would take more parameters to describe these sorts of surfaces. This is a possible reason why the FI model fit the Kern *et al.* [20] data set better with the strongly asymmetric surfaces predicted in tetanic stimulus and laryngoscopy, the two models with the highest difference in AICs between the Minto and FI models. Overall, the FI model fit the clinical interaction study data slightly better than the Minto model while allowing statistically testable parameters for curve shape properties. We believe this adds significantly to the ability to generate interaction surfaces as a means for evaluating interactions in a statistical, quantifiable manner.

References

1. Liu RH: Potential synergy of phytochemicals in cancer prevention: Mechanism of action. J Nutr 2004; 134:3479S–85S

2. Murphy JT, Tucker JM, Davis C, Berger FG: Raltitrexed increases tumorigenesis as a single agent yet exhibits anti-tumor synergy with 5-fluoruracil in ApcMin/+ mice. Cancer Biol Ther 2004; 3:1169–76

3. Room MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M: Antitumor effect of nutrient synergy on human osteosarcoma cells U-sOS, MNNG-HOS and Ewing's sarcoma SK-ES. Oncol Rep 2005; 13:253–7

4. Gherardi MM, Ramirez JC, Esteban M: IL-12 and IL-18 act in synergy to clear vaccinia virus infection: Involvement of innate and adaptive components of the immune system. J Gen Virol 2003; 84:1961–72

5. Gouwy M, Stuyf S, Catusse J, Proost P, Van Damme J: Synergy between proinflammatory ligands of G protein-coupled receptors in neutrophil activation and migration. J Gen Virol 2003; 76:185–94

6. Rand KH, Houck HJ: Synergy of daptomycin with oxacillin and other beta-lactams against methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2004; 48:2871–5

7. Handslip PD, Dart AM, Davies BH: Intravenous salbutamol and aminophylline in asthma: A search for synergy. Thorax 1981; 36:741–4

8. Remington TL, Digiovine B: Long-acting beta-agonists: Anti-inflammatory properties and synergy with corticosteroids in asthma. Curr Opin Pulm Med 2005; 11:74–8

9. Berenbaum MC: What is synergy? Pharmacol Rev 1989; 41:93–141

10. Greco WR, Bravo G, Parsons JC: The search for synergy: A critical review from a response surface perspective. Pharmacol Rev 1995; 47:331–84

11. Kano Y, Akutsu M, Tsunoda S, Suzuki K, Yazawa Y, Furukawa Y: Schedule-dependent synergism and antagonism between raltitrexed ("Tomudex") and methotrexate in human colon cancer cell lines *in vitro*. Jpn J Cancer Res 2001; 92:72–82

12. Li RC, Schentag JJ, Nix DE: The fractional maximal effect method: A new way to characterize the effect of antibiotic combinations and other nonlinear pharmacodynamic interactions. Antimicrob Agents Chemother 1993; 37:523–31

13. Gennings C, Carter H Jr, Carney EW, Charles GD, Gollapudi BB, Carchman RA: A novel flexible approach for evaluating fixed ratio mixtures of full and partial agonists. Toxicol Sci 2004; 80:134–50

14. Charles GD, Gennings C, Zacharewski TR, Gollapudi BB, Carney EW: An approach for assessing estrogen receptor-mediated interactions in mixtures of three chemicals: A pilot study. Toxicol Sci 2002; 68:349–60

15. Minto CF, Schnider TW, Short TG, Gregg KM, Gentilini A, Shafer SL: Response surface for anesthetic drug interactions. ANESTHESIOLOGY 2000; 92:1603–16

16. Tam VH, Schilling AN, Lewis RE, Melnick DA, Boucher AN: Novel approach to characterization of combined pharmacodynamic effects of antimicrobial agents. Antimicrob Agents Chemother 2004; 48:4315–21

17. White DB, Slocum HK, Brun Y, Wrzosek C, Greco WR: A new nonlinear mixture response surface paradigm for the study of synergism: A three drug example. Curr Drug Metab 2003; 4:399–409

18. Germani M, Magni P, De Nicalo G, Pogessi I, Marsiglio A, Ballinari D, Rochetti M: *In vitro* cell growth pharmacodynamic studies: A new nonparametric approach to determining the relative importance of drug concentration and treatment time. Cancer Chemother Pharmacol 2003; 52:507–13

19. Kelly C, Rice J: Monotone smoothing with application to dose-response curves and the assessment of synergism. Biometrics 1990; 46:1071–85

20. Kern SE, Xie G, White JL, Egan TD: Opioid–hypnotic synergy: A response

Fig. 6. A plot of the Minto U_{50} function resulting from using the Minto model to estimate the flexible interaction model with 49 equally spaced points (not including 0 and 1) when the interaction is asymmetric. The Minto model isobole polynomial has the following parameters: $u_2 = -28.57$, $u_3 = 39.04$, $u_4 = -17.15$ (subscript describes order of the U_{50} polynomial parameter). The Minto model identifies two regions of antagonism [0, 0.502] and [0.775, 1] with one region of synergy [0.502, 0.775], where the flexible interaction model only identifies antagonism.

surface analysis of propofol–remifentanil pharmacodynamic interaction in volunteers. ANESTHESIOLOGY 2004; 100:1378–81

21. Short TG, Plummer JL, Chui PT: Hypnotic and anaesthetic interactions between midazolam, propofol and alfentanil. J Anesth 1992; 69:162–7

22. Short TG, Ho TY, Minto CF, Schnider TW, Shafer SL: Efficient trail design for eliciting a pharmacokinetic-pharmacodynamic model–based response surface describing the interaction between two intravenous anesthetic drugs. ANESTHESIOLOGY 2002; 96:400–8

23. Sakamoto Y, Ishiguro M, Kitagawa G: Akaike information criterion, Akaike Information Criterion Statistics. Edited by Hazewinkel M. Boston, D. Reidel Publishing, 1986, pp 56–89

24. Brent RP: An algorithm with guaranteed convergence for finding a zero of a function, Algorithms for Minimization without Derivatives. Edited by Forsythe G. Englewood Cliffs, New Jersey, Prentice Hall, 1973, pp 47–60

25. Ihaka R, Gentleman R: R: A language for data analysis and graphics. J Computational Graphical Statistics 1996; 5:299–314

26. Burnham KR, Anderson DR: Model Selection and Multimodel Inference, 2nd edition. New York, Springer, 2002, pp 70–2

27. Dahan A, Nieuwenhuijs D, Olofsen E, Sarton E, Romberg R, Tepperna L: Response surface modeling of alfentanil–sevoflurane interaction on cardiorespiratory control and Bispectral Index. ANESTHESIOLOGY 2001; 94:982–91

28. Niewenhuijs DJF, Olofsen E, Romberg R, Sarton E, Ward D, Engers F, Vuyk J, Morren R, Tepperna LJ, Dahan A: Response surface modeling of remifentanil–propofol interaction on cardiorespiratory control and Bispectral Index. ANESTHESIOLOGY 2003; 98:312–22