Appendix 1: Review of Common Drug Interaction Models

As mentioned in the introduction of the manuscript, isoboles are commonly used to analyze anesthetic drug interactions. Isoboles show dose combinations that result in equal effect.[1] The combination of two doses (d_1 and d_2) can be represented by a point on a graph, the axes of which are the doses of the individual drugs (Figure 1). If the interaction is additive, the isobole will be a straight line given by the equation:

$$\frac{d_1}{D_1} + \frac{d_2}{D_2} = 1$$

Equation (1.1)

where D_1 and D_2 are the isoeffective doses of the drug 1 and 2 when given alone. When the drugs in combination are more effective than expected (synergy), smaller amounts are needed to produce the effect, and the combination (d_1, d_2) is shifted toward the origin (figure 1, middle graph). Conversely, when the drugs in combination are less effective than expected (infra-additive), greater amounts are needed to produce the effect, and the combination (d_1, d_2) is shifted away from the origin (figure 1, lower graph).

Although an isobole clearly shows whether an interaction is additive, synergistic or antagonistic, it is often only determined for a single level of drug effect. In anesthesia, a common approach has been to determine the 50% isobole for a specific endpoint, such as preventing movement in response to an incision. Based on a single isobole, it is not possible to make inferences about other levels of drug effect that might be more clinically relevant (e.g., the 95% isobole) unless one also knows the steepness of the dose-response relationship.

Table 1 lists our proposed criteria for drug interaction models. The single isobole approach does not meet criteria 5 in Table 1, a critical shortcoming. It is possible to solve this problem by estimating isoboles for each level of response. This results in a huge number of parameters, which necessarily decreases the confidence about the individual parameters. Therefore, the "many isobole" approach does not meet criteria 3 in Table 1, as the parameters cannot be accurately estimated unless large studies are used.

When the drug effect is a probability of response, the response in the absence of drug, E_0, can be set to 0, and the maximum possible response, E_{max}, can be set to 1. Using this approach several investigators have modeled anesthetic drug interactions as extensions of the logistic regression model for a single drug.[2,3,4,5] In the single drug model, the natural logarithm of the odds ratio (the logit) is modeled as a linear function of drug concentration (C):

$$\text{logit} = \log(\text{odds ratio}) = \log\left(\frac{P}{1-P}\right) = \beta_0 + \beta_1 \cdot \log(C)$$

Equation (1.2)

where P is probability of effect, and β_0 and β_1 are estimated parameters. Alternatively, the probability of effect can be expressed as:

$$P = \frac{e^{\beta_0 + \beta_1 \cdot \log(C)}}{1 + e^{\beta_0 + \beta_1 \cdot \log(C)}}$$

Equation (1.3)

If $\beta_0 = -\gamma \cdot \log(C_{50})$ and $\beta_1 = \gamma$, then equations 1.2 and 1.3 are algebraically equivalent to the more intuitive and familiar sigmoidal relationship:

$$P = \frac{\left(\frac{C}{C_{50}}\right)^{\gamma}}{1+\left(\frac{C}{C_{50}}\right)^{\gamma}}$$

Equation (1.4)

where C_{50} is the drug concentration associated with a 50% probability of drug effect, and γ is the steepness of the concentration response relationship. This can be generalized to multiple drugs, either using the concentration or the log of the concentration. If concentration is used, then the interaction between two drugs is modeled as:

$$\log\left(\frac{P}{1-P}\right) = \beta_0 + \beta_1 \cdot C_1 + \beta_2 \cdot C_2$$

Equation (1.5)

where P is the probability of effect, β_0, β_1, and β_2 are estimated parameters, and C_1 and C_2 are two drug concentrations. Solving equation 1.5 for $P = 0.5$ yields the 50% isobole, given by the straight line:

$$\frac{C_1}{-\beta_0/\beta_1} + \frac{C_2}{-\beta_0/\beta_2} = 1$$

Equation (1.6)

where $-\beta_0/\beta 1 = C_{50,1}$ and $-\beta_0/\beta_2 = C_{50,2}$. Equation 1.5 has several limitations. If only one drug is present ($C_2 = 0$), then:

$$P = \frac{e^{\beta_0+\beta_1\cdot C_1}}{1+e^{\beta_0+\beta_1\cdot C_1}}$$

Equation (1.7)

which is inconsistent with the single drug model (Equation 1.3) because *C* cannot be transformed to be *Log*(*C*), as required by the single drug model. Thus, equation 1.5 does not meet criteria 7 in Table 1. When both drugs are absent ($C_1 = C_2 = 0$), then:

$$P = \frac{e^{\beta_0}}{1+e^{\beta_0}}$$

Equation (1.8)

which predicts drug effect *even when no drug is present* (unless $\beta_0 = -\infty$), thus violating criteria 6 of Table 1. Although equation (1.5) generates the correct linear isobole at the 50% level, for all other probabilities the isoboles are flawed, violating criteria 1 in Table 1.

An alternative approach for two drugs is to take the natural logarithm of the drug concentrations:[3,5]

$$\log\left(\frac{P}{1-P}\right) = \beta_0 + \beta_1\cdot\log(C_1) + \beta_2\cdot\log(C_2)$$

Equation (1.9)

Solving for $P = 0.5$ yields the 50% isobole:

$$e^{\beta_0} \cdot C_1^{\beta_1} \cdot C_2^{\beta_2} = 1$$

Equation (1.10)

Equation 1.10 *always* suggests profound synergy, because it is the equation for an hyperbola. The isobole predicted in equation 1.10 necessarily bends profoundly in the middle in order to reach infinity as it approaches each axis. One way of dealing with the problems of this model is to add an arbitrary constant to one or both drug concentrations.[3] This arbitrarily chosen constant has an enormous influence on the other parameter estimates, but has no pharmacodynamic meaning, and even with this constant Equation 1.10 is still an hyperbola.

An alternative approach, used for over 50 years, is to model drug interactions based on pharmacological principals.[6] An example is provided by Greco,[7] who has adapted the guidelines of Berenbaum[8] and others, to develop an interaction model for two drugs based on a sigmoidal E_{max} model and the following isobole constraint:

$$\frac{C_1}{C_{50,1}} + \frac{C_2}{C_{50,2}} + \alpha \cdot \frac{C_1}{C_{50,1}} \cdot \frac{C_2}{C_{50,2}} = 1$$

Equation (1.11)

This equation is one of five different isobole models illustrated by Machado and Robinson[9] based on a single "non-additivity parameter", α. Such models can describe both synergy and infra-additivity on the value of α. This parameter allows gradations in the shape of the isobole. Positive values of α suggest synergy, and negative values suggest infra-additivity. Although these isobole constraints enable objective statistical evaluation of drug interactions, they are insufficiently flexible and cannot be used to model interactions that result in a maximum effect greater than either drug alone, nor can they describe asymmetric isoboles. Thus, this approach does not satisfy criteria 4 in Table 1.

Appendix 2: Mathematics of Triple Interaction Model

As described in the Anesthesiology manuscript, the two drug model can be readily expanded to show the interaction of more than two drugs. In the case of three drugs (A, B and C) the proportion of each drug present can be expressed by θ_A, θ_B and θ_C, where:

$$\theta_A = \frac{U_A}{U_A + U_B + U_C}\ ,\ \theta_B = \frac{U_B}{U_A + U_B + U_C}\ \text{and}\ \theta_C = \frac{U_C}{U_A + U_B + U_C}$$

Equation (2.1)

We can completely define the ratio of three drugs from just two of these ratios because $\theta_A + \theta_B + \theta_C = 1$. As described in the Anesthesiology manuscript, we will use θ_B and θ_C. We assume that for any fixed value of θ_B and θ_C, there is a sigmoidal relationship between concentration and response. In other words, if the three drugs could be administered to the effect site in an exactly fixed proportion, they would demonstrate a sigmoidal "concentration" vs. response relationship, where the "concentration" was the sum of the three normalized concentrations. This is precisely the notion that underlies the two drug model. The equation for the three drug model is thus:

$$E = E_0 + (E_{\max}(\theta_B, \theta_C) - E_0)\frac{\left(\frac{U_A + U_B + U_C}{U_{50}(\theta_B, \theta_C)}\right)^{\gamma(\theta_B, \theta_C)}}{1 + \left(\frac{U_A + U_B + U_C}{U_{50}(\theta_B, \theta_C)}\right)^{\gamma(\theta_B, \theta_C)}}$$

Equation (2.2)

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As in the two drug interaction model, the basic concept behind the three drug model is that for any fixed ratio of three drugs, A, B, or C, there is a simple sigmoid concentration-response relationship. However, the parameters of this relationship are no longer a function of a single ratio, as in the two drug model, but are a function of the ratio of each individual drug to the total. Thus each of the parameters in the three drug interaction model, E_{max} (θ_B, θ_C), U_{50} (θ_B, θ_C), and γ (θ_B, θ_C), are each functions of θ_B and θ_C The challenge for the three drug model is to develop these functions of θ_B and θ_C that meet the following criteria.

1) If A, B, or C, is zero, then the model should resolve mathematically to the interaction model for remaining two drugs.

2) If there are no interactions between A, B, and C, then the model should resolve to a simplex plane defined by the values for A alone, B alone, and C alone. In the case of the model for U_{50}, these points are, by definition, all at U_{50} = 1. This is shown in the top graph of Figure 2, the "simple additive interaction" model for U_{50} for three drugs.

3) Consider a sham experiment in which drugs B and C are actually the same drug, a drug that shows synergy with drug A. In this sham experiment, the interaction of A with any combination of B and C should yield the same interaction as the interaction between A and B, and the interaction between A and C. This is seen in the middle graph of Figure 2.

4) The model should be symmetrical with regards to A, B, and C. In other words, it should meet the above criteria regardless of which drug is assigned as A, B, and C.

For our purposes, we will only present a model of quadratic interactions, i.e., a model where each interaction is, at most, defined by a parabola. Higher order models are possible, but graphical analysis

suggests that it is difficult to identify the parameters of higher order models from typical experimental data.

Our proposed model is as follows. Any general parameter *P* can be expressed as a function of the composition vector (θ_A, θ_B, θ_C) according to the following model:

$$P = q_0 + q_B \cdot \theta_B + q_C \cdot \theta_C + q_{BB} \cdot \theta_B^2 + q_{CC} \cdot \theta_C^2 + q_{BC} \cdot \theta_B \cdot \theta_C + q_{ABC} \cdot \theta_A \cdot \theta_B \cdot \theta_C$$

Equation (2.3)

This model has the following characteristics:

1. the model is polynomial, therefore smooth and infinitely differentiable;
2. the model is a function of the θ_B and θ_C, because $\theta_A = 1 - \theta_B - \theta_C$. θ_A is explicitly expressed in the last term for clarity. Note that the q_{ABC} is a "triple" interaction term, and the product $q_{ABC} \cdot \theta_A \cdot \theta_B \cdot \theta_C$ vanishes at all the three edges of the simplex, i.e. when we consider just two interacting drugs;
3. we can substitute the last term, $q_{ABC} \cdot \theta_A \cdot \theta_B \cdot \theta_C$,with the more general expression, $\theta_A \cdot \theta_B \cdot \theta_C \cdot f(\theta_A \cdot \theta_B \cdot \theta_C)$, $f(.)$ being any general function of the composition vector; this substitutive term satisfies the same property of vanishing on the axes and enables more flexibility with the choice of $f(.)$;

Equation 2.3 was developed by assuming that for every pair of drugs the interaction model is known and has the form:

$$P_{AB} = P_A + \left(P_B - P_A - \beta_{2,AB}\right)\cdot \theta_B + \beta_{2,AB}\cdot \theta_B^2$$

Equation (2.4)

$$P_{AC} = P_A + \left(P_C - P_A - \beta_{2,AC}\right)\cdot \theta_C + \beta_{2,AC}\cdot \theta_C^2$$

Equation (2.5)

$$P_{BC} = P_B + \left(P_C - P_B - \beta_{2,BC}\right)\cdot \theta_C + \beta_{2,BC}\cdot \theta_C^2$$

Equation (2.6)

Where P_A, P_B, and P_C are the parameters of the single drugs when given alone, and $\beta_{2,AB}$, $\beta_{2,AC}$ and $\beta_{2,BC}$ are the coefficients for the three paired interactions. If one imposes the surface (2.3) to become each one of the three interaction pairs (2.4) to (2.6) when $\theta_C = 0$, $\theta_B = 0$, $\theta_A = 0$ respectively, the following set of constraints are obtained:

$$P_A = q_0$$

Equation (2.7)

$$P_B = q_0 + q_B + q_{BB}$$

Equation (2.8)

$$P_C = q_0 + q_C + q_{CC}$$

Equation (2.9)

$$\beta_{2,AB} = q_{BB}$$

Equation (2.10)

$$\beta_{2,AC} = q_{CC}$$

Equation (2.11)

$$\beta_{2,BC} = q_{CC} + q_{BB} - q_{BC}$$

Equation (2.12)

We obtain 6 linear equations. This was expected since we know *a priori* 6 parameters of the model, namely the single drug parameters (P_A, P_B, P_C) and the coefficients of the paired interactions ($\beta_{2,AB}$, $\beta_{2,AC}$, $\beta_{2,BC}$).These constraints can be inverted to calculate the coefficients of the general model(2.3):

$$q_0 = P_A$$

Equation (2.13)

$$q_B = P_B - P_A - \beta_{2,AB}$$

Equation (2.14)

$$q_C = P_C - P_A - \beta_{2,AC}$$

Equation (2.15)

$$q_{BB} = \beta_{2,AB}$$

Equation (2.16)

$$q_{cc} = \beta_{2,AC}$$

Equation (2.17)

$$q_{BC} = \beta_{2,AB} + \beta_{2,AC} - \beta_{2,BC}$$

Equation (2.18)

In this model, there is only one free parameter to describe the three drug interaction, namely the constant term q_{ABC} ; this can be regarded as a generalization of the two interaction case with a parabolic interaction, in which the only free parameter to describe the A-B drug interaction was $\beta_{2,AB}$. If one wishes to describe more complex types of three drug interactions one can simply re-express the last term in equation (2.3) as observed earlier without perturbing the consistency conditions [(2.7) to (2.12)] or [(2.13) to (2.18)].

Model (2.3) resolves to a simple parabolic interaction for the remaining two drugs if θ_A, θ_B, or θ_C = 0, satisfying condition 1 of our 3 drug model. If there is no interaction for $U_{50}(\theta_B,\theta_C)$, q_B, q_C, q_{BB}, q_{CC}, q_{BC} and q_{ABC} are 0. In this case, the model (2.3) reduces to $P=q_0=1$, as seen in the top graph of Figure 2. This satisfies condition 2. If B and C are the same drug, then $q_B = q_C$, $q_{BB} = q_{CC}$, and $q_{BC} = q_{ABC} = 0$. This generates the shape shown in the middle graph of Figure 2, and satisfies condition 3. We observe, without showing the algebra, that the model is completely symmetrical with respect to the three drugs. We mention this only because symmetry proved to be a major hurdle in the development of the three-drug model.

Figures

Figure 1

Isobolograms; (A) additivity, (B) synergy, (C) infra-additivity. D_1 and D_2 are isoeffective doses of two drugs given alone. The administration of the two drugs in combination (d_1,d_2) results in the same effect. If D_1 and D_2 are the D_{50} doses, in each case the line represents the 50% isobole.

Figure 2.

This figure introduces the three drug parameter surface, as well as the three drug axes seen at the base. Representative surfaces for U_{50} as a function of θ_B and θ_C. The three corners of the triangular surface represent the value of $U_{50}(\theta_B, \theta_C)$ for drug A alone, drug B alone and drug C alone. The three edges represent the value of $U_{50}(\theta_B, \theta_C)$ for the paired combinations for A and B (labeled θ_{AB}), A and C (labeled θ_{AC}), and B and C (labeled θ_{BC}). The surface represents the value of $U_{50}(\theta_B, \theta_C)$ for the three drug combination. The top graph shows additive interaction for all three drugs. The middle graph shows synergistic interaction between drug A and drug B, and between drug A and drug C, but additive interaction between drug B and drug C. The bottom graph shows synergistic interactions between all three drugs.

Figure 1:

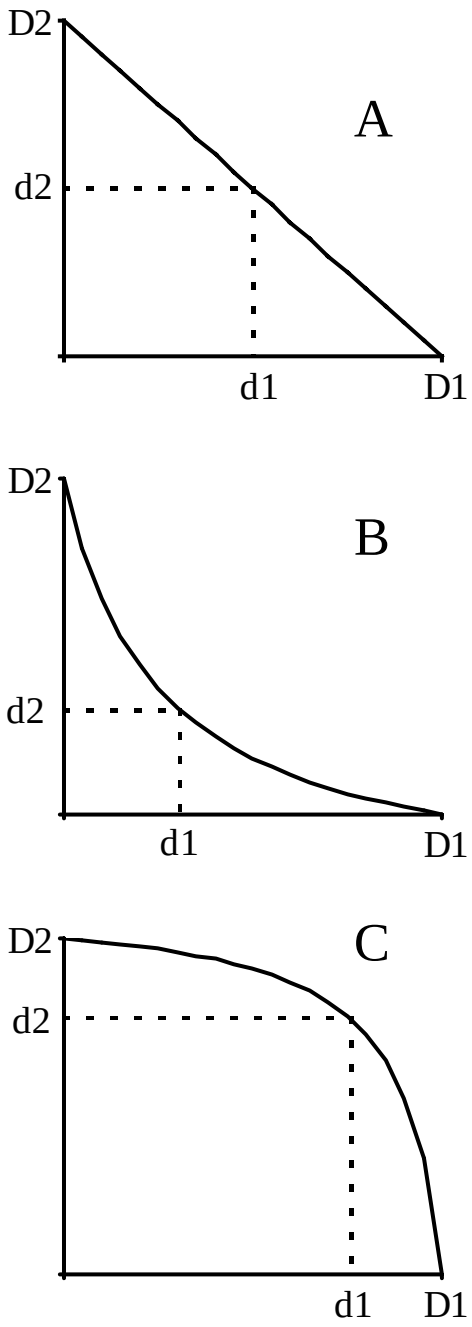

Figure 2:

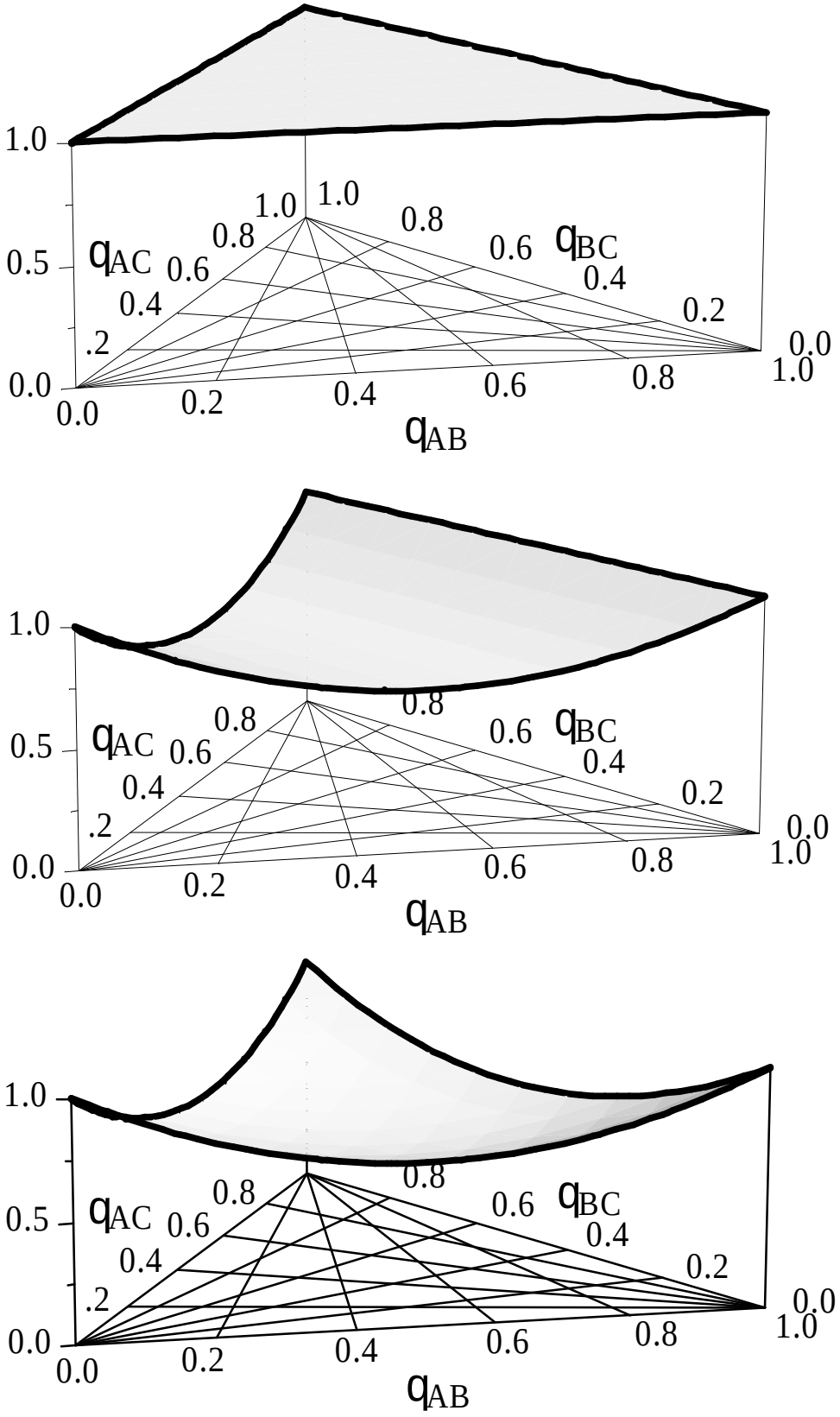

Table 1:

Characteristics of an Ideal Pharmacodynamic Interaction Model

1. The interaction model is consistent with prior mathematical proofs (e.g. additive isobole, Equation 1).
2. The interaction model is equally valid for any measure of drug exposure, such as dose, plasma concentration, tissue concentration, or effect site concentration.
3. The parameters of the interaction model can be accurately estimated from studies of reasonable size.
4. Interaction parameters provide flexibility in the concentration-response relationship of the interacting drugs, permitting assessment of additive, synergistic and antagonistic interactions, and interactions when the interacting drugs differ in the steepness of the concentration-response relationship or the maximum effect of the drugs.
5. The interaction model predicts the response over the entire clinical range of doses or concentrations for one, two or three drugs.
6. The interaction model predicts no drug effect, when no drugs are present.
7. If one of the drugs in the interaction model is not present, the model reduces to the correct model for the remaining drugs.

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