

ASSESSMENT OF INTERACTION OF BIOLOGICALLY ACTIVE AGENTS BY MEANS OF THE ISOBOLE APPROACH: FUNDAMENTAL ASSUMPTIONS AND RECENT DEVELOPMENTS

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Abstract

There is a widespread confusion about concepts, methods and terminology for evaluating a possible interaction between biologically active agents. According to our view this is primarily due to the fact that the meaning and limits of applicability of the methods used are not known precisely enough. The aim of this contribution is to take a step towards a better understanding of the isobole approach. It is widely used for the assessment of interaction of biologically active agents. Nevertheless, objections as to the general validity of this method for all types of dose-response relations, be they linear or non-linear, similar or dissimilar, monotonic or non-monotonic, and for all mechanisms of action continue to exist. Arguments are presented which support the view that this method can be applied to all types of dose-response relations. Combining the classical isobole method with response surface modeling and computer graphics leads to powerful new methods for evaluating interaction between biologically active agents. In particular, so-called zero interaction response surfaces are proposed which show no interaction, according to the isobole approach, for the complete dose range. In addition to the (one-dimensional) index of interaction and to the two-dimensional isobologram these surfaces represent a novel three-dimensional approach for analyzing combination experiments. Furthermore, interaction as defined by the isobole criterion is compared to other definitions of interaction (effect summation criterion, independence criterion). Finally, the non-mechanistic isobole approach is related to mechanistic models of interaction of biologically active agents.

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Key words: Combined effects - Dose response - Isobole approach

Introduction

Studies of the combined action of biologically active agents become increasingly important in many branches of biomedical and environmental research. Nevertheless the confusion about concepts, terminology and methods for evaluating a possible interaction continues to exist (Berenbaum 1989; Greco et al 1990; Pösch, 1991; Prichard and Shipman Jr 1990; Sühnel 1990; Sühnel 1992; Sühnel in press b). According to our view the primary reason for this unfortunate situation is that the limits of applicability of the various procedures used are not known precisely enough. The usual approach is to calculate from the single-agent effects an expected combination effect for the case of 'no interaction', whatever this means. A deviation of the observed combination

effect from the expected effect then indicates interaction. Almost all of the different procedures for evaluating a possible interaction between biologically active agents can be traced back to only three basic criteria: the effect summation criterion, the independence criterion, the isobole criterion. All these criteria are based on different definitions of interaction. This means that analyzing one and the same experimental data set by means of different criteria may lead to different results. In other words, protagonists of one criterion may claim that the criteria which are not preferred by them do not represent a correct definition of no interaction. Therefore, it is certainly one of the basic goals of theoretical work in the field of combination experiments to try to reach consensus on the most appropriate definition of interaction.

It is further important to point out that the criteria mentioned are empirical tools. This means that concentrations, effects and empirical concentration-effect relations but no mechanistic information is required. This is more an advantage than a disadvantage. It is an advantage because these approaches can be applied to systems for which mechanistic information is not available. Further, given there is some knowledge on the mechanism the results do not depend on the usual progressive modifications of the mechanism. This is also the reason why it can be expected that these approaches can be applied in rather different fields of research with possibly completely different mechanisms of action. On the other hand, empirical evaluation procedures approaches do not explain, of course, what is really going on. Therefore, an interesting new development is to combine empirical and mechanistic models (Sühnel, in preparation).

Terminology

If different terms are used for one and the same item or phenomenon or if one and the same term is applied to different experimental situations this may be rather confusing. This is exactly the case in the field of combination experiments. On the other hand, this is not a basic problem and one should not worry about it too much given the terms used are precisely defined. It is more important to scrutinize the various definitions of interaction and the methods based on them.

The terms synergism and antagonism are widely used to indicate deviations both from the effect summation criterion and from the independence criterion (Pöch 1991). Combination effects which obey the isobole criterion for no interaction are often called additive (Unkelbach and Pöch 1988; Pöch 1991). On the other hand, it was proposed to use the terms zero interaction, synergism and antagonism also in this case (Berenbaum 1985; Berenbaum 1989). The reason for this proposal was that according to the isobole criterion addition of effects is only correct for linear dose-response relations and not for other types of dose-response curves. In order to avoid this difficulty but to retain the term additive one speaks occasionally of a dose-additive interaction, because the isobole equation represents a sum of fractional doses (Pöch 1991). On the other hand, this criterion has the same mathematical structure both for the cases of interaction and no interaction.

In this paper the following simple terminology is used: The terms interaction and independence are used in a synonymous manner. If two agents act independently, then it is said that there is no interaction between them. Zero interaction or no interaction or independence are used whenever one of the criteria of no interaction is obeyed. Effects larger than expected are called synergistic and effects smaller than expected antagonistic. Note that in this sense the so-called independence criterion represents only one possible definition of independence or interaction. This terminology requires, of course, that in all cases the criterion used for the evaluation of a possible interaction has to be mentioned.

Criteria for Defining Interaction

This work is mainly concerned with the isobole criterion. It is therefore beyond its scope to discuss all criteria in detail. However, a short description is appropriate.

Effect summation criterion

The action of a varying dose d_A of agent A on the combination effect E_{AB} is given by $\delta E_{AB}/\delta d_A$. Of course, for agent B the corresponding effect is described by $\delta E_{AB}/\delta d_B$. On the other hand, the action of d_B on the action of d_A on E_{AB} is given by the

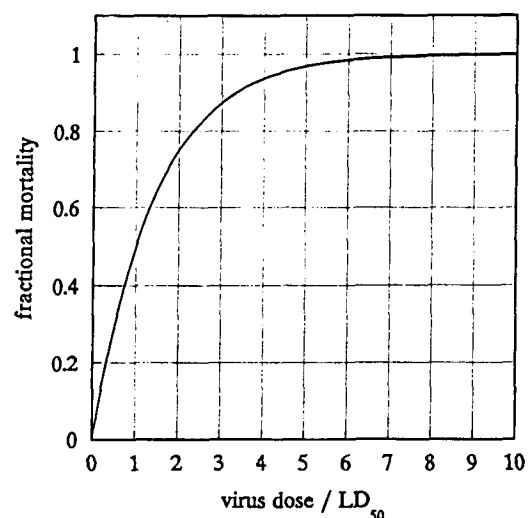


Fig. 1. Dose-response relation between virus dose and fractional mortality [$E(d)=1 - \exp(-d \ln 2)$]; all graphs were generated using GRAFTOOL, version 3.3, 3-D Visions Corporation, Redondo Beach, CA, USA]

second partial derivative $\delta E_{AB}/\delta d_A \delta d_B$. It is obvious, that if there is no action of d_B on d_A on E_{AB} one can speak of no interaction. In this case the second partial derivative is zero and this condition is only fulfilled if the combination effect equals the sum of single-agent effects (Drury 1980).

$$E_{AB}^{ES}(d_A, d_B) = E_A(d_A) + E_B(d_B) \quad (1)$$

Therefore this criterion is called effect summation. E_{AB}^{ES} stands for the effect calculated according to this criterion. Note that for the calculation of the expected effect only the single-agent effects are required. This criterion is certainly correct for linear dose-response curves. On the other hand, one runs into difficulties for curves which approach or display maxima or minima. This is shown in Figure 1. Here, a typical dose-response curve for the action of a certain virus on fractional mortality of a group of laboratory animals is shown. Almost all dose-response relations of this type have the shape shown. They simply differ in the absolute amount of virus particles representing 1 LD₅₀. 1 LD₅₀ is the virus dose leading to a fractional mortality of 50%. Let us now assume that a group of laboratory animals is infected both by one virus which leads, when given alone, to 80% mortality, and by another virus which leads to, say 60% mortality, when given alone. Effect summation then predicts a fractional mortality of 140%, which makes no sense, of course. According to this criterion the maximum possible response would in this case be classified as interactive, which is simply due to the fact that the animals available are limited to 100%. Note that the very same reasoning applies to any other target, say enzymes or receptors and so on. It is, of course, a certain type of interaction if one agent acts on targets which are then no longer available for the other agent. However, this is not the type of interaction one usually thinks about. Therefore, it makes sense to look for other definitions of interaction which overcome this difficulty. Both the independence criterion and the isobole criterion try to do this, however, in a different manner.

Independence Criterion

According to the independence criterion (Pöch 1991) no interaction is defined by

$$E_{AB}^{IN}(d_A, d_B) = E_A(d_A) + E_B(d_B) - E_A(d_A)E_B(d_B) \quad (2a)$$

$$= E_A(d_A) + (1 - E_A(d_A)) E_B(d_B) \quad (2b)$$

(fractional effects: $0 \leq E \leq 1$)

E_{AB}^{IN} is the calculated combination effect according to the independence criterion. There is a simple rationale for this criterion. If agent A displays an effect E_A , then agent B can only act on the remaining effect range $(1 - E_A)$, see equation (2b). Unkelbach and Pöch (1988) have pointed out that according to the independence criterion the relative or net effect of one agent is independent of the other. This property is easily obvious from equation (2b). The relative or net effect of E_B as defined by Unkelbach and Pöch (1988) is obtained if the second term in equation (2b) is divided by the effect range $(1 - E_A)$. This gives, of course, a constant value E_B . On the other hand, the absolute contribution of E_B to the combination effect is scaled by E_A , equation (2b). In so far it is also correct to say that the contribution of E_B to E_{AB}^{IN} is dependent on E_A .

As already noted the independence criterion tries to define no interaction in a useful manner also for non-linear dose-response curves which approach maximum values. According to our view it is rather surprising that this is done without taking into account the specific shapes of the dose-response relations. In other words, it is not taken into account if these relations are steep or flat. In order to discuss this problem let us assume that two agents have the rather extreme dose-response relations shown in Figure 2. Up to an effect of 0.8 the dose-response relations are definitely linear and one need not worry about the maximum value. Let us further assume that two doses of the two agents are combined which exhibit a fractional effect of 0.4 when used alone. It is obvious that in this case the combination effect should be 0.8 given there is no interaction. The independence criterion, however, leads to an effect of $0.4 + 0.4 - 0.16 = 0.64$. On the other hand, both the effect-summation and the isobole approach give the expected value of 0.8.

Another problem refers to the sham combination of two amounts of one and the same agent. Let us assume that two amounts of one and the same agent having any of the dose-response curves shown in Figure 2 are combined. One can then calculate the expected effect according to the independence

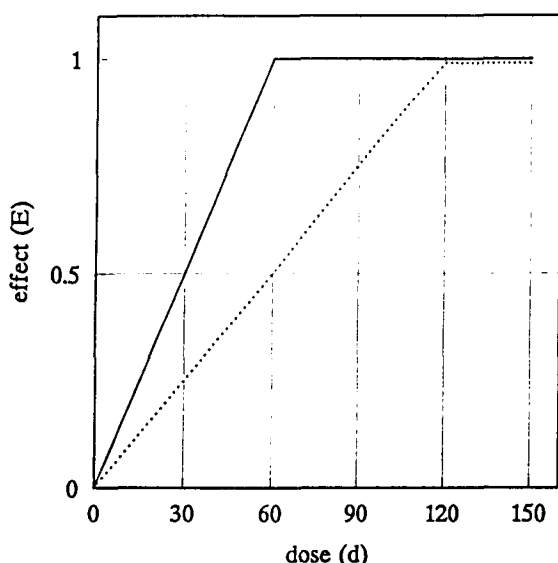


Fig. 2. Dose-response relations (solid line: $E(d) = (0.5/30)d$ for $d \leq 60$, $E(d) = 1$ for $d > 60$; dotted line: $E(d) = (0.5/60)d$ for $d \leq 120$, $E(d) = 1$ for $d > 120$)

criterion equation (2). On the other hand, one can add the doses and directly read off the effect from the dose-response curve, Figure 2. Given that both single-agent effects are 0.35 it turns out that the independence criterion leads to an expected effect of 0.5775, whereas direct reading off the effect from the dose-response curve gives an effect of 0.7. It was shown that the results are only identical for exponential dose-response curves (Berenbaum 1989; Sühnel in press a). In other words, except for exponential dose-response curves the 'combination' of two amounts of one and the same agent is classified as interactive according to the independence criterion.

Isobole Criterion

Within the isobole criterion the reference point of no interaction is defined by the 'sham combination' of two amounts of one and the same agent (Loewe 1953; Berenbaum 1989). In other words, according to this criterion the 'sham combination' of one and the same agent is per definition zero-interactive. No interaction within the isobole method is defined by means of the isobole equation (3)

$$\sum_i (d_i / D_i) = I \quad (3)$$

In this equation d_A, d_B are the combination doses and

D_A, D_B are the single-agent doses which produce the same magnitude of effect as the combination. The index of interaction I is 1 for zero interaction and smaller than one for synergism or larger than one for antagonism. It is very important to note that contrary to the other criteria one needs some information on the shapes of dose-response relations even for the evaluation of only one dose combination. This means that the relationship between the single-agent and the combination effect is dependent on the type of the dose-response relation. For linear relations the effect-summation criterion is obtained and for exponential dose-response relations the independence criterion. Other dose-response curves lead to other relations. There are, of course, also objections to the isobole approach. They are discussed in more detail in the following.

Which Definition of Interaction is the Correct One?

It would be very satisfying if there were a simple answer to this question. The long-lasting discussion on this topic shows, however, that this is not possible, so far. According to our view the main reason for this fact is that meaning and limits of the different criteria are not known precisely enough. This contribution is mainly concerned with the isobole approach. It provides new arguments and describes new methodological developments and thus tries to describe the isobole criterion more precisely.

The Isobole Approach

Recent Developments and Application to Experimental Data

The term isobole approach as used here stands for all evaluation procedures based on the isobole criterion. The usual procedures are either the calculation of the index of interaction according to the isobole equation or the construction of isobolograms. An isobologram represents a two-dimensional diagram in which on the coordinate axis the doses of agents A and B are plotted and which displays lines of equal biological effect, the isoboles, see Figure 8. Given linear dose scales are used, linear isoboles represent dose combinations with an interaction index of 1. Any deviation from linear isoboles indicates a deviation from the case of no interaction as defined by the isobole criterion. In addition, we have recently

proposed several three-dimensional evaluation procedures based on the isobole approach (Sühnel in press a). The most powerful is probably the application of so-called *zero interaction response surfaces*. These are surfaces which have interaction indices $I=1$ for all dose combinations or linear isoboles for all effect levels. By means of response surface modeling and current computer graphics this procedure is now easily amenable to routine application. A further three-dimensional procedure is the *interaction function*. This is a three-dimensional generalization of the index of interaction. An application of this procedure is described by Baumgart et al (1991). Occasionally, *difference response surfaces* are useful representations (Prichard and Shipman Jr 1990; Sühnel in press a). Note that all these procedures are based on one and the same criterion and thus give identical results when applied correctly.

The construction of zero interaction response surfaces is in the following described in more detail. Starting point is the isobole equation (3) with $I=1$. As already noted this equation defines zero interaction or no interaction according to the isobole criterion. One can now easily combine this equation with mathematical expressions for single-agent dose-response relations. The quantities D_A and D_B represent doses of single agents A and B which exhibit the same effect as the combination. Note that per definition

$$E_{AB}(d_A, d_B) = E_A(D_A) = E_B(D_B) \quad (4)$$

holds. Recasting the dose-response relations after the doses, inserting the corresponding expression into the denominator of the isobole equation and replacing both the single-agent effects E_A and E_B by the effect of the combination E_{AB} thus gives a mathematical expression which interrelates the combination effect E_{AB} with d_A, d_B . This expression describes the combination effect as function of the doses under the restraint that the interaction index I equals 1 for all dose combinations. This is nothing more than the mathematical definition of a zero interaction response surface. We have derived the corresponding expression for various well-known dose-response relations (Sühnel in press a). In some cases the expressions obtained are very simple. In some cases implicit equations are obtained which have to be solved by iteration. We have therefore written the

computer program COMBITOOL which solves these equations. The data obtained in this manner can then directly be imported by any computer graphics software capable of displaying three-dimensional graphics. We recommend the graphical analysis system GRAFTOOL distributed by 3-D Visions Corporation, Redondo Beach, CA, USA. Two examples for zero interaction response surfaces are to be given here. For linear dose-response relations

$$E(d) = \alpha d \quad (5)$$

the corresponding zero interaction response surface is given by

$$E_{AB}^{IB}(d_A, d_B) = \alpha_A d_A + \alpha_B d_B. \quad (6)$$

Greek letters represent parameters of the dose-response relations and E_{AB}^{IB} is the combination effect calculated according to the isobole criterion. Equation (6) corresponds to the effect summation criterion, equation (1). As already noted, according to the isobole criterion, effect summation is correct for linear dose-response curves. The widely used power-function dose-response relation

$$E(d) = (d/\alpha)^\mu \quad (7)$$

leads to

$$\{d_A/[\alpha_A(E_{AB}^{IB})^{1/\mu_A}]\} + \{d_B/[\alpha_B(E_{AB}^{IB})^{1/\mu_B}]\} = 1 \quad (8)$$

For $\mu_A = \mu_B = \mu$ this implicit equation simplifies to

$$E_{AB}^{IB} = [(d_A/\alpha_A) + (d_B/\alpha_B)]^\mu \quad (9)$$

One application of the zero interaction response surface to experimental data is shown in Figures 3 and 4. Olsson (1986) has reported data on olfactory mixtures of pyridine and dimethyldisulfide (DMDS). In a first step the single-agent dose-response relations have to be determined. In the field of olfaction and taste research it is common practice to use power-function dose-response relations, equations (10) and (11).

$$E_{\text{pyridine}} = (d_{\text{pyridine}}/2.19)^{0.44} \quad (10)$$

$$E_{\text{DMDS}} = (d_{\text{DMDS}}/0.14)^{0.34} \quad (11)$$

The corresponding zero interaction response surface

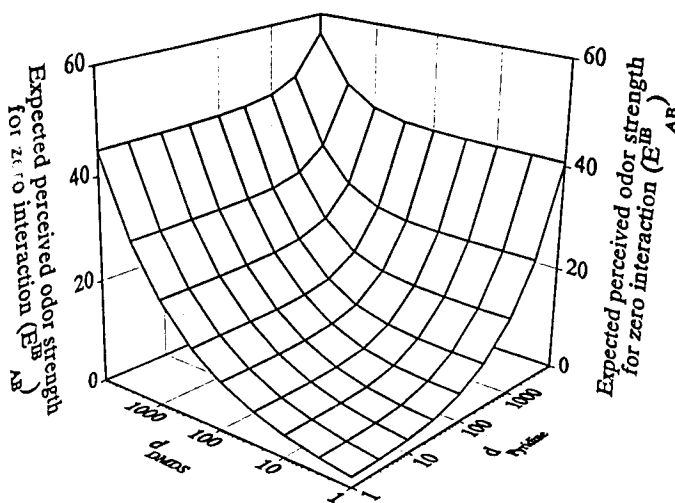


Fig. 3. Zero interaction response surface E_{AB}^{IB} (expected perceived odor strength for the case of zero interaction according to the isobole approach) for psychophysical data for the mixture of pyridine and dimethyldisulfide (DMDS) reported by Olsson (1986) (E_{AB}^{IB} was calculated by means of equation (12); d in ppb)

is then given by equation (12), see equation (8),

$$\frac{d_{\text{pyridine}}}{2.19(E_{AB}^{IB})^{1/0.44}} + \frac{d_{\text{DMDS}}}{0.14(E_{AB}^{IB})^{1/0.34}} = 1 \quad (12)$$

This surface is displayed in Figure 3. Figure 4 shows the difference between the experimental data and the effects obtained from the zero interaction response surface. As compared to the absolute effect values the deviation is rather small. This means that the mixture of pyridine and DMDS is zero-interactive in the whole dose range. It is important to point out that the construction of a zero interaction response surface requires only single-agent dose-response curves. Our experience is that a direct fitting of surfaces to combination effects is a rather difficult task (Gennings et al 1990; Prichard and Shipman Jr 1990; Sühnel 1990). The results are very dependent on the dose design and on the mathematical function used. All these difficulties can be avoided by means of zero interaction response surfaces. They can easily be applied to experimental systems for which only few combination experiments in different dose range are available.

Note that the isobole approach can be applied to all possible dose-effect relations. For example, one

can easily evaluate combinations of X-rays and chemicals or temperature effects and so on.

In environmental research it is often difficult to obtain dose-response relations. One would thus prefer to have an evaluation procedure at hand which simply requires the two single-agent effects and a combination effect. Contrary to the independence and to the effect summation criterion this does not suffice for the isobole criterion. One definitely needs some information on the single-agent dose relations even though it is not necessary to have a complete analytical expression of this relation.

In the following an example is discussed which shows how the isobole approach can be applied if no complete dose-response relations are available. Fisher (1992) has studied the joint toxic action of insecticides and of chlordimeform (CDF) on arthropodes. His evaluation procedure was the following: He determined the LD_{50} values of the insecticide alone and of the insecticide combined with CDF. The interaction parameter RS was then calculated according to equation (13).

$$RS = 10^M \quad M = m_1 - m_2 \quad (13)$$

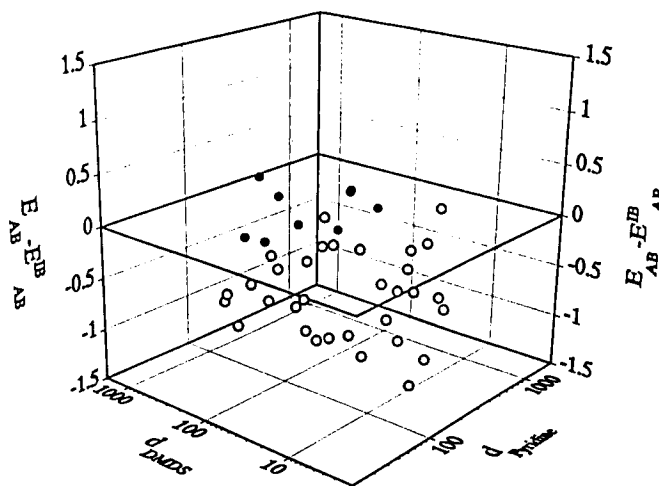


Fig. 4. Difference $E_{AB} - E_{AB}^{IB}$ for olfactory mixtures of pyridine and DMDS (Olsson 1986). (E_{AB} stands for the experimental data and E_{AB}^{IB} for the expected effect for zero interaction calculated according to equation (12); full circles indicate positive values and empty circles negative values; the values below the zero plane (empty circles) are shown in all cases even if they are covered by the zero plane; d in ppb)

m_1 - log LD₅₀ for insecticide alone
 m_2 - log LD₅₀ for insecticide and CDF
 (chlordimeform)

According to Fisher (1992) values of $RS < 1$ indicate antagonism, values of $1 < RS < 2$ marginal interaction and values of $RS > 2$ an more than additive response. The CDF dose used in the combination experiment was nontoxic (0% mortality). If CDF does not display an effect at all when used alone then the procedure used by Fisher is identical to the isobole approach. The isobole equation for this experiment is given by

$$d_{\text{insecticide}}/D_{\text{insecticide}} + d_{\text{CDF}}/D_{\text{CDF}} = 1 \quad (14)$$

In equation (14) $D_{\text{insecticide}}$ and D_{CDF} stands for the LD₅₀ values of the insecticide and of CDF and $d_{\text{insecticide}}$ and d_{CDF} are the doses which in combination produce an effect of 50%. If CDF does not display an effect at all, then the quantity C_{CDF} in the denominator of the second term of the isobole equation has to be set to infinity. In this case the term disappears and the evaluation is simply a comparison of the amounts of the insecticide when used alone and in combination which produce an effect of 50%. This is exactly the approach used by Fisher (1992). If, however, CDF is toxic at higher concentrations then this approach is not correct according to the isobole criterion. In this case the LD₅₀ values of both the insecticide and CDF have to be determined and inserted in the isobole equation (14). Then the second term in the equation is also relevant and this may lead to different results as obtained by Fisher. The effect will be especially marked if the dose-response curve of the toxic action of CDF increases sharply after the dose used in the combination experiment. It will almost not affect the results if the LD₅₀ value of CDF is high as compared to the concentration used in the combination experiment.

Objections to the Isobole Approach

The main objections to the isobole approach raised over the years are:

It does not define 'no interaction' in an appropriate manner but rather describes 'additivity' which is a certain type of interaction (Unkelbach and Pöch 1988).

It is only applicable to certain types of dose-

response curves. Some authors claim that this approach can only be applied to linear dose-response curves (Steel and Peckham 1979). Others assume that the approach can be applied to non-linear relations given they are similar (Loewe 1953; Unkelbach and Pöch 1988; Pöch 1991). Two dose-response curves are called similar if they are superimposable simply by changing the linear scale of the dose-axis. Furthermore, it is well-known that one will run into difficulties with non-monotonic dose-response curves (Berenbaum 1985; Berenbaum 1989).

It can only be applied to agents competing for the same binding site (Pöch 1991).

It is not possible to discuss all these arguments in detail. However, a few arguments will be presented here.

The first one refers to the derivation of the isobole equation. It seems that most researchers are not well aware of the arguments used in deriving the isobole equation. On the other hand, these arguments are essential for answering the questions posed above. Berenbaum (1989) has given a derivation of this equation and we have added a few new arguments (Sühnel, in preparation).

The basic assumption of the isobole approach is that 'no interaction' is defined by the sham combination of two amounts of one and the same agent. Now, the question is whether or not a sham combination can mimic a real combination in all relevant properties. Relevant properties are the combination doses d_A , d_B , the combination effect $E_{AB}(d_A, d_B)$ and the single-agent doses D_A , D_B , which exhibit the same magnitude of effect as the combination. That means it holds equation (4). Note that it is not necessary to mimic the effects of single-agent doses, when given alone: $E_A(d_A)$, $E_B(d_B)$. Agent B is mimicked by a (D_B/D_A) fold dilution of agent A. This material is called A'. In Figure 5 two dissimilar dose-response curves are shown. A dose 30 (dimensionless quantities) of agent A produces an effect of 0.5 and a dose 60 of agent B produces an effect of 0.5, too. To mimic B by A agent A has to be diluted (D_B/D_A) fold, that means in this case twofold. A' contains (D_A/D_B) amounts of A. In the case shown this corresponds to half of the concentration of A. It holds

$$E_B(D_B) = E_{A'}[(D_A/D_B)D_B] = E_A(D_A) \quad (15)$$

This means A' mimicks B in the relevant properties

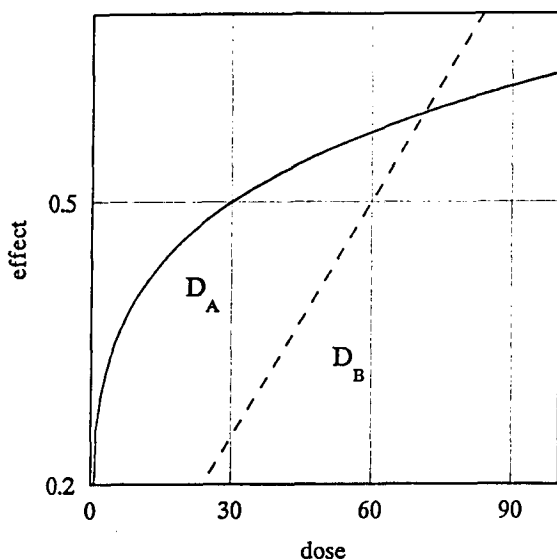


Fig. 5. Dissimilar dose-response relations for agents A and B (solid line, agent A, $E_A=(d_A/960)^{0.2}$; dashed line, agent B, $E_B=(d_B/120)$; D_A and D_B are the doses which produce an effect of 0.5)

D_B and $E_B(D_B)$. It is not necessary to mimic B in $E_B(d_B)$. This is only done for similar dose-response relations. Note, however, that this is one point of controversy. The sham combination is iso-effective with the real combination if the following relations are fulfilled:

$$E_{AB}(d_A \text{ of A} + d_B \text{ of B}) = E_{AA'}(d_A \text{ of A} + (D_A/D_B)d_B \text{ of A}) = E_A(D_A \text{ of A}) \quad (16)$$

This leads for monotonic relations between doses and effects to

$$d_A + (D_A/D_B)d_B = D_A \quad (17)$$

Recasting gives

$$(d_A/D_A) + (d_B/D_B) = 1 \quad (18)$$

This is the isobole equation for two agents which can easily be generalized to more than two agents. This derivation leads to the following conclusions:

The derivation does not require any assumptions on the shapes of dose-response relations (except for monotonicity) or on the mechanism of interaction. Therefore the isobole approach can be applied to all types of dose-response relations and if mechanistic information is not available.

In addition, we have recently shown that the isobole approach can also be applied to non-

monotonic dose-response relations given the zero interaction response surfaces described above are used (Sühnel in press a; Sühnel in preparation). In Figure 6, a surface of this type is shown. Even though the corresponding isobologram is not shown one can easily see that it consists exclusively of straight lines. The single agent-dose response relations used is

$$E(d) = \alpha d - \beta d^2 \quad (d \leq (\alpha/\beta)) \quad (19)$$

This relation has a maximum effect $E_{max}=(\alpha^2/4\beta)$ at dose $d_{max}=(\alpha/2\beta)$. Assuming $\alpha_A=\alpha_B=\alpha$ and $\beta_A=\beta_B=\beta$ leads to the following expression for the zero interaction response surface E_{AB}^{IB}

$$E_{AB}^{IB}(d_A, d_B) = \alpha(d_A + d_B) - \beta(d_A^2 + 2d_A d_B + d_B^2) \quad (d_A + d_B \leq 130) \quad (20a)$$

$$E_{AB}^{IB}(d_A, d_B) = 0 \quad (d_A + d_B > 130) \quad (20b)$$

Straightforward application of the isobole equation leads to wrong results in this case. For example, an interaction of $I < 1$ indicates synergism in the low-dose region and antagonism in the high-dose region. In other words, the interpretation has to be reversed in these two regions. Given the dose-response curve does not display this simple pattern this may lead to difficulties. As already noted they can be overcome by

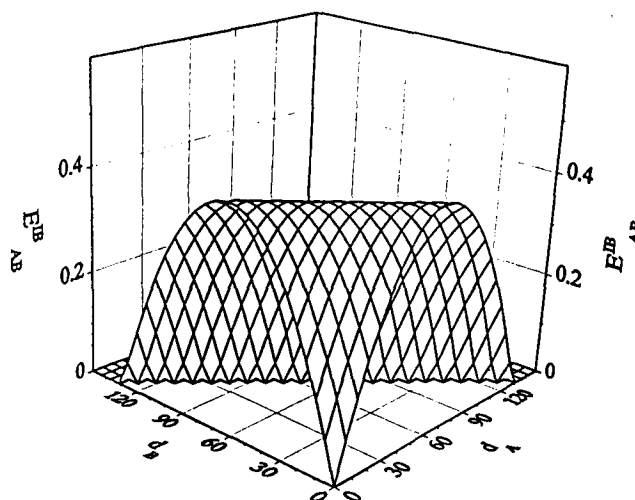


Fig. 6. Zero interaction response surface E_{AB}^{IB} for two agents with the non-monotonic dose-response relations (single agent dose-response relations according to equation (19); zero interaction response surface according to equation (20); $\alpha=0.013$, $\beta=0.0001$)

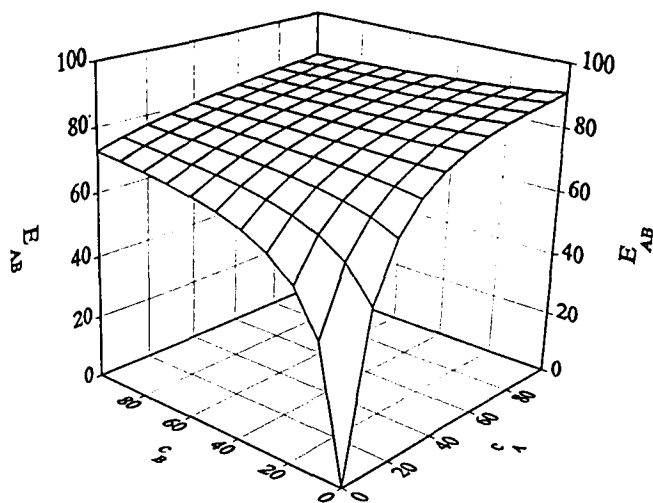


Fig. 7. Response surface according to equation (22); $K_A=K_B=10, E_{max}^A=100, E_{max}^B=80$

means of the zero interaction response surface.

Relation Between the Empirical Isobole Approach and Mechanistic Models

The zero interaction response surface describes the dependence of the combination effect on the doses and gives thus an expression for $E_{AB}(d_A, d_B)$. Mechanistic models yield analogous expressions which can thus directly be compared. As already noted the combination of empirical and mechanistic

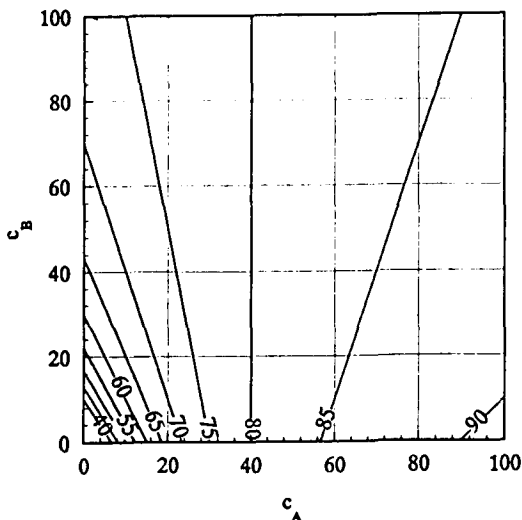


Fig. 8. Isobologram for the response surface shown in Figure 7

models seems to be a very promising approach. One example is to be discussed in the following.

In three seminal papers Ariëns and coworkers (1956) proposed several mechanistic models for the interaction of biologically active agents with receptor systems. Based on mass-action considerations it was assumed that the concentration dependence of effect E of a single agent can be described by equation (21). According to Ariëns et al (1956) in the following the concentration c is used instead of the dose d .

$$E(c) = E_{max} c / [K + c] \tag{21}$$

In equation (21) c is the concentration, K is the dissociation constant for the complex between the biologically active agent and the receptor and E_{max} is the maximum value of the effect. Assuming that two agents A and B compete for one and the same receptor system Ariëns et al (1956) arrived at equation (22).

$$E_{AB}(c_A, c_B) = [E_{max}^A K_B c_A + E_{max}^B K_A c_B] / [K_A c_B + K_B c_A + K_A K_B] \tag{22}$$

This the so-called model of competitive interaction. On the other hand, the procedure for deriving an expression for the zero interaction response surface described above using the single-agent concentration-response relation of equation (21) leads exactly to the same equation (22). In other words, the zero interaction response surface obtained without any mechanistic information and the competitive interaction surface obtained without any information on the isobole approach are identical. In a certain sense this comparison yields a mechanistic explanation of the empirical isobole approach. In Figures 7 and 8 the corresponding response surface and the isobologram are shown. Upon closer inspection it turns out that the comparison between the mechanistic model of competitive interaction and the empirical isobole approach is slightly more involved. For $E_{max}^A = E_{max}^B$ the complete competitive interaction surface is identical with the zero interaction surface. For $E_{max}^A \neq E_{max}^B$ this surface is only zero interactive up to the lower E_{max} value, see the isobologram in Figure 8. This behaviour can easily be understood mechanistically. For E_{AB} values above the lower E_{max} value the corresponding agent can no longer make a positive contribution to the combination effect, does, however, still occupy

receptor sites.

According to our view it is very likely that the approach described in the last part of this work will lead to new insights in the field of combination experiments. For example, it would very interesting to construct mechanistic models which give combination effects identical to the other criteria for assessing interaction between biologically active agents.

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