

Zero Interaction Response Surfaces, Interaction Functions and Difference Response Surfaces for Combinations of Biologically Active Agents

J. Sühnel¹⁾

Summary

In the field of combination experiments there is widespread confusion over definitions, terminology and methods for the evaluation of interaction between biologically active agents. According to our view the widely used isobole approach is the method of choice. In this contribution it is shown how the combination of the classical isobole approach with response surface modeling and computer graphics leads to powerful new methods for the assessment of interaction of biologically active agents. In particular, zero interaction response surfaces, difference response surfaces and interaction functions are proposed. Zero interaction response surfaces represent surfaces which display zero interaction in the whole dose range. Difference response surfaces display the difference between an actual response surface and the corresponding zero interaction response surface. Interaction functions are a generalization of the index of interaction, which describe the dose dependence of this quantity.

Zusammenfassung

Null-Wechselwirkungsflächen, Wechselwirkungsfunktionen und Differenzwirkungsflächen für Kombinationen biologisch aktiver Verbindungen

Im Bereich der Analyse von Kombinationswirkungen existiert eine weitverbreitete Konfusion im Hinblick auf Definitionen, Terminologie und Methoden. Nach unserer Überzeugung ist der vielfach angewendete Isobolen-Ansatz die Methode der Wahl. In diesem Beitrag wird gezeigt, wie die Kombination der klassischen Isobolen-Methode mit Wirkungsflächenmodellierung und Computergraphik zu leistungsfähigen neuen Verfahren zur Bewertung von Kombinationswirkungen führt. Im einzelnen werden Null-Wechselwirkungsflächen, Differenzwirkungsflächen und Wechselwirkungsfunktionen vorgeschlagen. Null-Wechselwirkungsflächen stellen Wirkungsflächen dar, die im gesamten Dosisbereich keine Wechselwirkung zeigen. Differenzwirkungsflächen beschreiben die Differenz zwischen einer Wirkungsfläche und der entsprechenden Null-Wechselwirkungsfläche. Wechselwirkungsfunktionen stellen eine Verallgemeinerung des Wechselwirkungsindex dar, die die Dosisabhängigkeit dieser Größe beschreiben.

Key words: *Biologically active agents, methods for analysis of combination effects · Difference response surfaces · Interaction functions · Zero interaction response surfaces*

1. Introduction

Interactions between drugs, carcinogens, environmental pollutants, physical stimuli, etc. are of fundamental importance and practical interest in many branches of biomedical research. Further progress in this field requires the profound evaluation of type and extent of interaction. Unfortunately, there is widespread confusion about definitions, terminology and methods. It is beyond the scope of this paper to compare the different approaches in de-

tail, for a review see [1]. However, for a better understanding of the underlying philosophy of the methods proposed in this paper a short discussion is appropriate. For the assessment of a possible interaction between biologically active agents, it is necessary to have criteria at hand which predict the effect of a combination from the effects of single agents for the case of no interaction. Any deviation from the expected effect then indicates an interaction. Upon closer examination of the various methods used it turns out that a great deal of them can be traced back to only two basic concepts usually called independence and additivity [2, 3]. M. C. Berenbaum's point of view is adopted that the widely used isobole approach, which is based on the concept of additivity, is the method of choice for the evaluation of a possible interaction between biologically active agents [1, 4]. To date, however, the application of this method has been primarily devoted to the evaluation of a possible interaction for single dose combinations or single effect levels. On the other hand, response surface modeling is a very useful

¹⁾ Present address at the end of this paper.

method for analyzing effects of combined biologically active agents. The modeling procedure may be performed by either fitting mathematical functions of the complete surface to experimental data [5], using a piecewise fitting procedure with spline functions [6], or by simply connecting the data points with straight lines [7]. Further, there are attempts to use response surface modeling not only for the representation of the response surface but also for the evaluation of possible interactions between the agents under study [6–9]. The classical isobole approach can easily be interrelated with the response surfaces, as the contour plots of these surfaces represent nothing more than the corresponding isobolograms. If isobolograms do not display simple patterns, one would prefer to use approaches which directly indicate type and extent of interaction in the dose range under study. The objective of this work is to propose such procedures by combining the classical isobole method with response surface modeling and computer graphics. In this contribution the focus is on the development of novel methods. Applications will be described in further papers.

2. The isobole method

As already noted in the introduction the isobole method is based on a concept usually called additivity [1–3]. The application of the isobole method requires data for agents used alone and in combination at equi-effective levels [1, 4]. According to the isobole equation

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

the index of interaction I can be calculated. The quantities d_A, d_B are the doses of agents A and B used in combination and D_A, D_B are the doses of agents A and B that would individually produce the same magnitude of effect as the combination. The isobole equation can be easily extended to more than two agents. The discussion in this paper is, however, restricted to the case of two agents. Eq. (1) with $I = 1$ represents the mathematical criterion for the case of no interaction. Berenbaum has shown that eq. (1) holds irrespective of the shapes of dose-response relations of single agents, provided these shapes are monotonic [1, 4]. As will be shown in the next section a simple addition of effects for the case of no interaction is only correct for linear dose-response curves. Therefore, Berenbaum has proposed to avoid the term additivity and to use instead zero interaction for the case of no interaction. In this terminology, which is also used throughout this work, interaction indices greater than 1 indicate antagonism, smaller than 1 synergism and equal to 1 zero interaction. Note, however, that another terminology for the very same situation is additivity for $I = 1$, supraadditivity for $I < 1$ and subadditivity for $I > 1$ [2, 3]. Hence, readers who prefer this latter terminology should in the following automatically replace zero interaction by additivity, synergism by supraadditivity and antagonism by subadditivity. A plot of one or several lines connecting different dose combinations which all produce the same magnitude of biological effect (isoboles) is called an isobologram. If the shape of an isobole is concave-up the interaction is synergistic, if it is concave-down the interaction is antagonistic and zero interaction is indicated by straight lines (for linear dose scales). Note, however, that a nonlinear isobole may have different values of I for different dose combinations. Therefore, it is useful to know the values of I for all dose combinations in the dose range under study. This is discussed in more detail in section 4.

The second basic criterion for the evaluation of an interaction between biologically active agents is the concept of independence [2, 3]. In this case the effect of a combination of two agents for the case of independence E_{AB}^i is calculated according to eq. (2)

$$E_{AB}^i = E_A + E_B - E_A E_B \quad (2)$$

Whereas combination effects which obey eq. (2) are called independent, larger than expected effects are occasionally also called synergistic and effects smaller than expected antagonistic. Note that eqs. (1) and (2) lead only to identical results if one of the agents does not show an effect when used alone or if the single-agent dose-response relations are exponential. Even though a more or less phenomenological description of the differences of the concepts of additivity and independence has been published a more detailed investigation of the meaning and limits of these two concepts remains to be done [3]. It is especially surprising that according to eq. (2) the expected effect for the case of independence should be independent of the steepness of single-agent dose-response curves. Moreover, application of eq. (2) may lead to synergism or antagonism when two amounts of one and the same agent are 'combined' (again except for the case of exponential dose-response curves). On the other hand, the isobole approach never yields synergism or antagonism for a sham 'combination' of one and the same agent. Note that often the terms synergism (antagonism) are used if one agent increases (decreases) the effect of the second agent [2]. This is identical to the definitions described above if one of the agents does not show an effect when used alone. However, if both of the agents combined show an effect when used alone this definition leads to different results.

3. Zero interaction response surfaces

How can the isobole equation be combined with the dose-response relations of single agents? The quantities D_A and D_B represent doses of single agents A and B which exhibit the same effect as the combination. 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 E_{AB} with d_A, d_B and I . One can now proceed in two different ways. If the interaction index I is set equal to 1 an expression for the so-called zero interaction response surface E_{AB}^0 is obtained. This is a response surface for which the corresponding isobologram shows exclusively straight lines or, in other words, for which all dose combinations have an interaction index of 1. On the other hand, one can replace E_{AB} by any response surface function with the same dose-response relations of single agents and obtain in this way an expression for a generalization of the interaction index, the so-called interaction function $I(d_A, d_B)$. This latter possibility is discussed in more detail in the next section. If in the isobole equation both the doses in the denominator and in the numerator are replaced and I is set to 1 one arrives at a relationship between E_{AB}^0 and E_A, E_B . In the following, for nine widely used dose-response relations the corresponding equations are presented and in a few cases graphs of zero interaction response surfaces are shown. A few of the following equations were already reported by Berenbaum [1]. The discussion is restricted to combinations with dose-response relations of the same type with possibly different parameters. Note, however, that at least implicit expressions can also be obtained for combinations with different types of dose-response relations. For the sake of brevity the functions $E_{AB}^0(d_A, d_B)$, $E_A(d_A)$, $E_B(d_B)$ are written in shorthand notation E_{AB}^0, E_A, E_B . In some cases it is more appropriate to use instead of effect E survival S defined by $E = 1 - S$. Greek letters stand for the parameters of the dose-response relations. All effects E and doses d are given in dimensionless quantities. The remaining part of this section is focused on a compilation of equations. A discussion of the meaning of

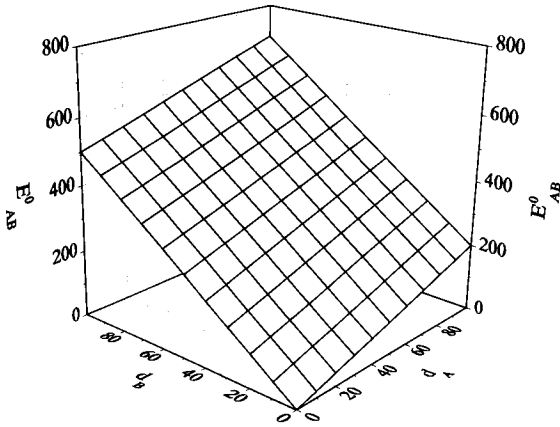


Fig. 1: Zero interaction response surface for linear dose-response relations, eq. (4); $E_{AB}^0 = 2 d_A + 5 d_B$. All graphs were generated using the GRAF-TOOL software package, version 3.3, distributed by the 3-D Visions Corporations.

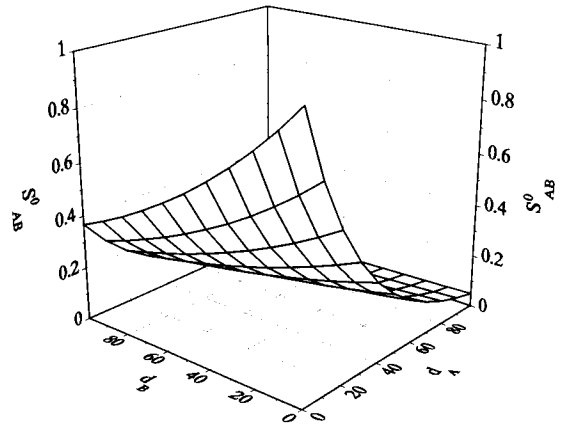


Fig. 2: Zero interaction response surface for exponential dose-response relations, eq. (11); $S_{AB}^0 = \exp(-0.03 d_A) \exp(-0.01 d_B)$.

parameters and on origin and applications of the dose-response relations used will not be given. The lines marked by an asterisk indicate particular parameters relationships.

a) Linear dose-response relation

$$E(d) = \alpha d \quad (3)$$

$$E_{AB}^0 = \alpha_A d_A + \alpha_B d_B \quad (4)$$

$$E_{AB}^0 = E_A + E_B \quad (5)$$

For linear dose response relations the effects can be simply added to obtain the expected effect for zero interaction. Note that this is the only case for which this is correct.

Therefore, as already noted, M. C. Berenbaum has suggested that one should avoid the term additivity and rather use zero interaction [1]. A zero interaction response surface for linear dose-response relations of single agents is shown in Fig. 1.

b) Weibull dose-response relation including the simple exponential relation

$$E(d) = 1 - \exp[-(\alpha d)^\mu]; S(d) = \exp[-(\alpha d)^\mu] \quad (6)$$

$$[\alpha_A d_A / (-\ln S_{AB}^0)^{1/\mu_A}] + [\alpha_B d_B / (-\ln S_{AB}^0)^{1/\mu_B}] = 1 \quad (7)$$

$$(\ln S_A / \ln S_{AB}^0)^{1/\mu_A} + (\ln S_B / \ln S_{AB}^0)^{1/\mu_B} = 1 \quad (8)$$

These are implicit equations which have to be solved numerically by iteration. It is very important to note that this fact does not represent any difficulty in applying the concept of zero interaction response surfaces. By means of current computer graphics software data obtained numerically can be displayed very easily. For $\mu_A = \mu_B = \mu$ the following expression can be obtained.

$$* (\mu_A = \mu_B = \mu)$$

$$S_{AB}^0 = \exp[-(\alpha_A d_A + \alpha_B d_B)^\mu] \quad (9)$$

$$S_{AB}^0 = \exp[-\{(-\ln S_A)^{1/\mu} + (-\ln S_B)^{1/\mu}\}^\mu] \quad (10)$$

$$* (\mu_A = \mu_B = 1)$$

$$S_{AB}^0 = \exp[-\alpha_A d_A - \alpha_B d_B] \quad (11)$$

$$S_{AB}^0 = S_A S_B; E_{AB}^0 = E_A + E_B - E_A E_B \quad (12)$$

The widely used procedure of effect-multiplication, eq. (12), which is identical with the independence criterion, eq. (2), is in the light of the isobole approach only correct for exponential dose-response curves. In other words, the isobole approach and the concept of independence yield for exponential dose-response curves identical results. However, for other dose-response curves the results are different. In particular, the 'combination' of various doses of one and the same agent yields a synergistic or antagonistic interaction if eq. (12) is applied to non-exponential dose-response curves. A zero interaction response surface for $\mu_A = \mu_B = 1$ is shown in Fig. 2.

c) Multi-target dose-response relation

$$S(d) = 1 - [1 - \exp(-\alpha d)]^\mu; E(d) = [1 - \exp(-\alpha d)]^\mu \quad (13)$$

$$-\{\alpha_A d_A / \ln[1 - (E_{AB}^0)^{\mu_A}]\} - \{\alpha_B d_B / \ln[1 - (E_{AB}^0)^{\mu_B}]\} = 1 \quad (14)$$

$$\{\ln[1 - (E_A)^{\mu_A}] / \ln[1 - (E_{AB}^0)^{\mu_A}]\} + \{\ln[1 - (E_B)^{\mu_B}] / \ln[1 - (E_{AB}^0)^{\mu_B}]\} = 1 \quad (15)$$

$$* (\mu_A = \mu_B = \mu)$$

$$E_{AB}^0 = [1 - \exp(-\alpha_A d_A - \alpha_B d_B)]^\mu \quad (16)$$

$$E_{AB}^0 = [(E_A)^{1/\mu} + (E_B)^{1/\mu} - (E_A E_B)^{1/\mu}]^\mu \quad (17)$$

For $\mu_A = \mu_B = 1$ the results are, of course, identical to the previous dose-response relation.

d) Linear-log dose-response relation

$$E(d) = \alpha \log d + \beta \quad (18)$$

$$\{d_A / 10^{[(E_{AB}^0 - \beta_A) / \alpha_A]}\} + \{d_B / 10^{[(E_{AB}^0 - \beta_B) / \alpha_B]}\} = 1 \quad (19)$$

$$10^{[(E_A - E_{AB}^0) / \alpha_A]} + 10^{[(E_B - E_{AB}^0) / \alpha_B]} = 1 \quad (20)$$

$$* (\alpha_A = \alpha_B = \alpha; \beta_A = \beta_B = \beta)$$

$$E_{AB}^0 = \alpha \log(d_A + d_B) + \beta \quad (21)$$

$$E_{AB}^0 = \alpha \log\{10^{[(E_A - \beta) / \alpha]} + 10^{[(E_B - \beta) / \alpha]}\} + \beta = \alpha \log\{10^{[E_A / \alpha]} + 10^{[E_B / \alpha]}\} \quad (22)$$

e) Log-log dose-response relation

$$\log E(d) = \alpha \log d + \beta \quad (23)$$

$$\{d_A / 10^{[(\log E_{AB}^0 - \beta_A) / \alpha_A]}\} + \{d_B / 10^{[(\log E_{AB}^0 - \beta_B) / \alpha_B]}\} = 1 \quad (24)$$

$$10^{[(\log E_A - \log E_{AB}^0) / \alpha_A]} + 10^{[(\log E_B - \log E_{AB}^0) / \alpha_B]} = 1 \quad (25)$$

$$* (\alpha_A = \alpha_B = \alpha; \beta_A = \beta_B = \beta)$$

$$\log E_{AB}^0 = \alpha \log(d_A + d_B) + \beta \quad (26)$$

$$\log E_{AB}^0 = \alpha \log\{10^{[(\log E_A - \beta) / \alpha]} + 10^{[(\log E_B - \beta) / \alpha]}\} + \beta = \alpha \log\{10^{[\log E_A / \alpha]} + 10^{[\log E_B / \alpha]}\} \quad (27)$$

f) Median-effect dose-response relation

$$E(d) = d^\mu / (\alpha^\mu + d^\mu) \quad (28)$$

$$d_A / \{\alpha_A [E_{AB}^0 / (1 - E_{AB}^0)]^{1/\mu_A}\} + d_B / \{\alpha_B [E_{AB}^0 / (1 - E_{AB}^0)]^{1/\mu_B}\} = 1 \quad (29)$$

$$\{[E_A (1 - E_{AB}^0)] / [E_{AB}^0 (1 - E_A)]\}^{1/\mu_A} + \{[E_B (1 - E_{AB}^0)] / [E_{AB}^0 (1 - E_B)]\}^{1/\mu_B} = 1 \quad (30)$$

$$* (\mu_A = \mu_B = \mu)$$

$$E_{AB}^0 = \{[(d_A / \alpha_A) + (d_B / \alpha_B)]^\mu / \{1 + [(d_A / \alpha_A) + (d_B / \alpha_B)]^\mu\}\} \quad (31)$$

$$E_{AB}^0 = \{[E_A / (1 - E_A)]^{1/\mu} + [E_B / (1 - E_B)]^{1/\mu}\}^\mu / \{1 + [E_A / (1 - E_A)]^{1/\mu} + [E_B / (1 - E_B)]^{1/\mu}\} \quad (32)$$

$$* (\mu_A = \mu_B = 1)$$

$$E_{AB}^0 = (E_A + E_B - 2E_A E_B) / (1 - E_A E_B) \quad (33)$$

The expression of E_{AB}^0 in terms of d_A and d_B is identical to eq. (31) with $\mu = 1$.

g) Power-function dose-response relation

$$E(d) = (d/\alpha)^\mu \quad (34)$$

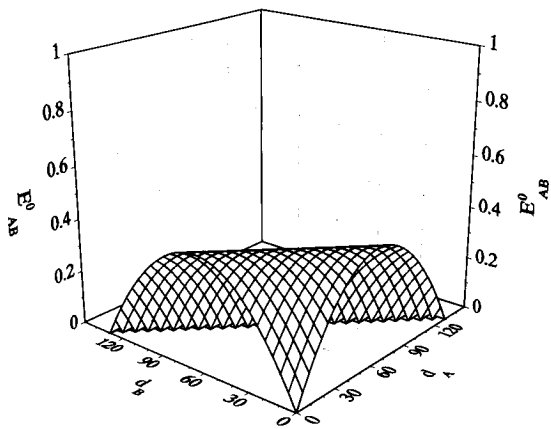


Fig. 3: Zero interaction response surface for polynomial dose-response relations, eq. (40); $E_{AB}^0 = 0.013(d_A + d_B) - 0.0001(d_A^2 + d_B^2 + 2d_A d_B)$.

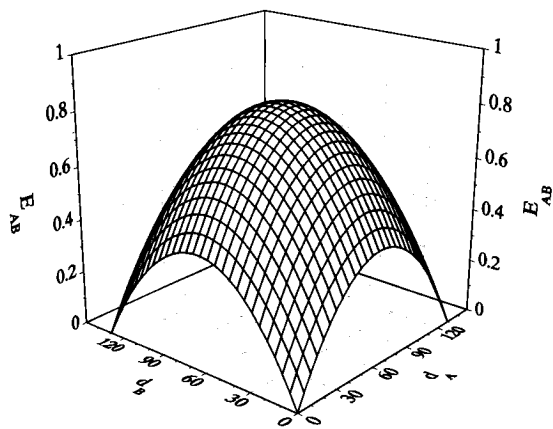


Fig. 4: Response surface for polynomial dose-response relations, eq. (41); $E_{AB} = 0.013(d_A + d_B) - 0.0001(d_A^2 + d_B^2)$.

This dose-response relation is widely used in the field of taste and olfaction research [10].

$$\{d_A/[\alpha_A (E_{AB}^0)^{1/\mu_A}]\} + \{d_B/[E_{AB}^0)^{1/\mu_B}]\} = 1 \quad (35)$$

$$(E_A/E_{AB}^0)^{1/\mu_A} + (E_B/E_{AB}^0)^{1/\mu_B} = 1 \quad (36)$$

$$* (\mu_A = \mu_B = \mu)$$

$$E_{AB}^0 = [(d_A/\alpha_A) + (d_B/\alpha_B)]^\mu \quad (37)$$

$$E_{AB}^0 = [(E_A)^{1/\mu} + (E_B)^{1/\mu}]^\mu \quad (38)$$

h) Polynomial dose-response relation

$$E(d) = \alpha d - \beta d^2 \quad (0 \leq d \leq (\alpha/\beta)) \quad (39)$$

This is a non-monotonic dose-response relation. The application of the isobole approach to non-monotonic dose-response relations requires a more detailed investigation which is mainly concerned with the invertibility of these relations and with the interpretation of particular values of I as synergistic or antagonistic. This is to be described in a subsequent paper. However, for the following particular case a simple equation for the zero interaction response surface can be obtained.

$$* (\alpha_A = \alpha_B = \alpha, \beta_A = \beta_B = \beta)$$

$$E_{AB}^0 = \alpha(d_A + d_B) - \beta(d_A^2 + d_B^2 + 2d_A d_B) \quad (40)$$

A graph of this surface is shown in Fig. 3. Note that in this case the expression for the zero interaction response surface contains a cross term. In contrast the simple addition of the dose-response relations of the single agents without a cross term results in a response surface and isobologram which is, as will be shown below, synergistic, eq. (41) and Fig. 4 and 5.

$$E_{AB} = \alpha(d_A + d_B) - \beta(d_A^2 + d_B^2) \quad (41)$$

According to this reasoning it is incorrect to assume that synergism or antagonism can be defined over the value and the sign of the coefficient connected with the cross term [11]. The single-agent dose-response relations for the response surfaces described by eqs. (40) and (41) and shown in Fig. 3 and 4 are exactly the same.

i) Logistic dose-response relation

$$E(d) = 1/[1 + \exp(-\alpha + \beta d - \tau d^2)] \quad (42)$$

This is again a non-monotonic dose-response relation and again for the following particular case a simple equation for E_{AB}^0 can be obtained.

$$* (\alpha_A = \alpha_B = \alpha; \beta_A = \beta_B = \beta; \tau_A = \tau_B = \tau)$$

$$E_{AB}^0 = 1/[1 + \exp\{-\alpha + \beta(d_A + d_B) - \tau(d_A^2 + d_B^2 + 2d_A d_B)\}] \quad (43)$$

After having presented mathematical expressions for zero interaction response surfaces and for the relation-

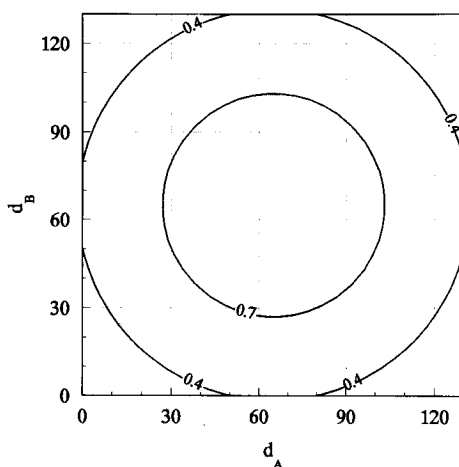


Fig. 5: Isobologram for the response surface shown in Fig. 4.

ships between single-agent effects and the combined effects in the case of no interaction for nine widely used dose-response relations it is important to note that the procedure described can easily be extended to any other dose-response relation. Zero interaction response surfaces can be generated from the single-agent dose-response relations alone. Therefore they can be applied to cases in which only a small number of dose combinations were studied. Deviations of experimental data points from the zero interaction response surface directly indicate synergism or antagonism.

4. Interaction functions and difference response surfaces

Type and extent of interaction may be dose-dependent. A combination of two compounds may act synergistically in one dose range and show antagonism in a different one. In other words, the interaction index I is, in principle, the value of a more general interaction function $\bar{I}(d_A, d_B)$ for a particular dose combination. This interaction function represents a surface analogous to the response surface and the corresponding contour plot displays lines of equal interaction (iso-interaction diagram). This latter diagram indicates directly regions of synergism, antagonism or zero interaction in the dose range under study. As already mentioned, the isobologram also yields information on a possible interaction. However, if

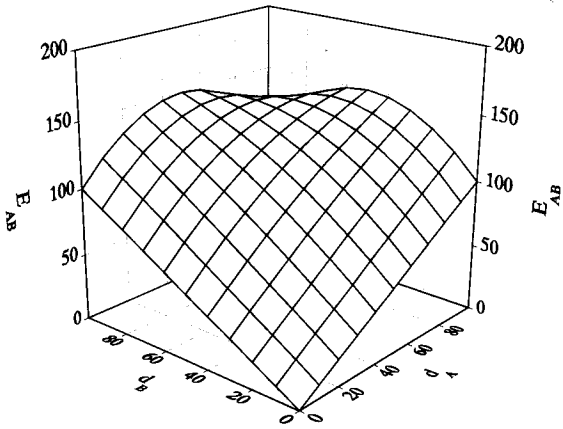


Fig. 6: Non-zero interaction response surface for linear dose response relations, eq. (44); $E_{AB} = d_A + d_B + 0.01d_A d_B - 0.000002d_A^2 d_B^2$.

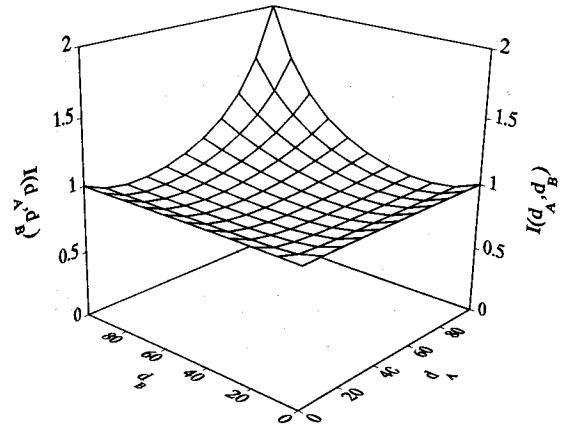


Fig. 8: Interaction function for the response surface shown in Fig. 6.

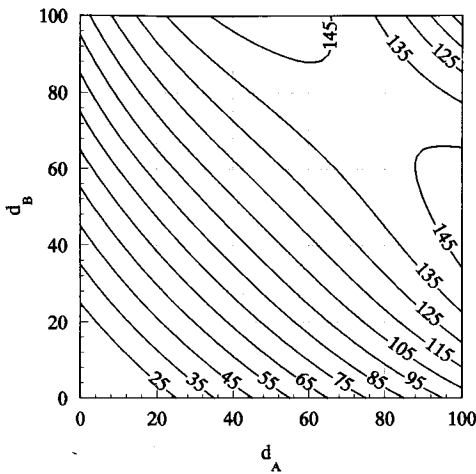


Fig. 7: Isobologram for the response surface shown in Fig. 6.

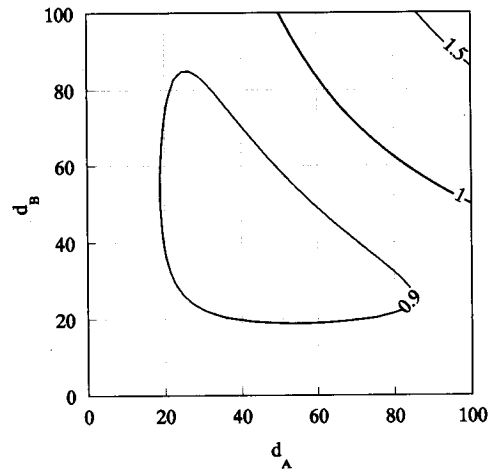


Fig. 9: Iso-interaction diagram for the response surface shown in Fig. 6.

isoboles do not display simple patterns or if logarithmic dose scales have to be used it may be rather troublesome to identify synergistic or antagonistic regions. This difficulty may be overcome by means of the interaction function and of the iso-interaction diagram. By means of the very same procedure adopted in [6], for example, spline functions can be fitted to the I values for all dose combinations. An example of the application of this approach is given in [8].

Here examples are discussed for which simple analytical expressions can be formulated for the interaction function. The response surface

$$E_{AB} = d_A + d_B + 0.01d_A d_B - 0.000002d_A^2 d_B^2 \quad (44)$$

shown in Fig. 6 and 7 is not a zero-interaction response surface. It has linear dose-response relations for the single agents. The corresponding zero-interaction surface is given by

$$E_{AB}^0 = d_A + d_B. \quad (45)$$

The interaction function for the surface of eq. (44) is given by

$$I(d_A, d_B) = (d_A + d_B) / (d_A + d_B + 0.01d_A d_B - 0.000002d_A^2 d_B^2). \quad (46)$$

A graphical representation of this function and of the iso-interaction diagram is shown in Fig. 8 and 9. Synergistic and antagonistic regions can easily be identified. The values of $I(d_A = 0, d_B)$ or $I(d_A, d_B = 0)$ and $I(0, 0)$ are equal to 1.

If the zero interaction response surface is known, synergistic or antagonistic regions can also be defined by means of the difference response surface $E_{AB} - E_{AB}^0$. Positive values of this difference indicate synergism and negative values antagonism. Zero interaction corresponds to a difference of zero. For the previous example this difference surface is shown in Fig. 10. In the corresponding contour plot, Fig. 11, the location of the zero line is identical to the line with $I = 1$ in the iso-interaction diagram, Fig. 9. Hence, both procedures yield identical results with regard to the location of synergistic or antagonistic regions.

When using the interaction index or the interaction function one may encounter several difficulties. It can happen that the effect of a certain dose combination is not reached by one of the agents alone within the dose range under study. If there is a chance that at higher doses of the single agent the effect of the combination will be reached, D_A or D_B have to be replaced by the dose producing the largest effect observed. This does not yield the correct value of I but rather an upper limit. Of course, if an analytical expression of the dose response-relation of single agents is known one could extrapolate to the dose required. However, one should never do that. One cannot be sure that the dose-response relation used is also correct in the extended dose range. Further, there are cases in which one can be sure that the effect cannot be reached by a single agent alone, in principle. In this case D_A or

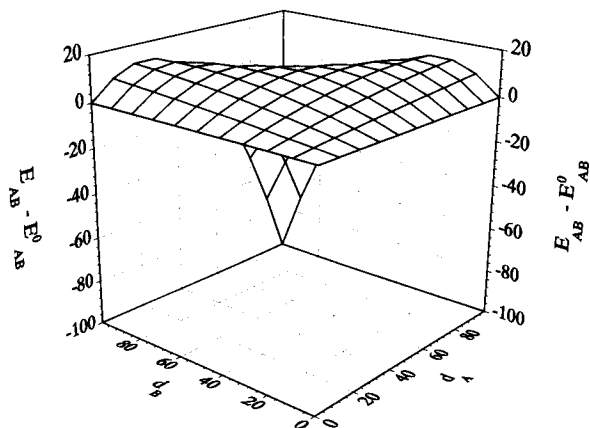


Fig. 10: Difference response surface for the response surface shown in Fig. 6 (solid lines — positive values including 0, dashed lines — negative values).

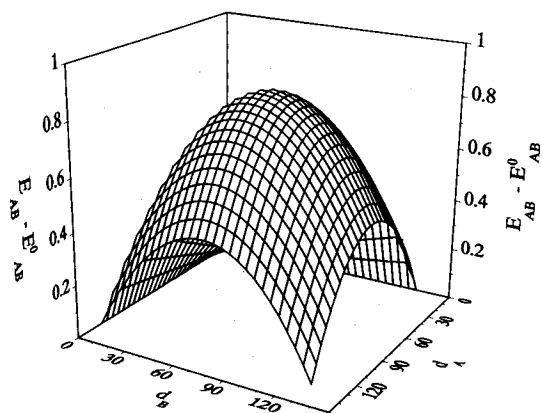


Fig. 12: Difference response for the response surface shown in Fig. 3.

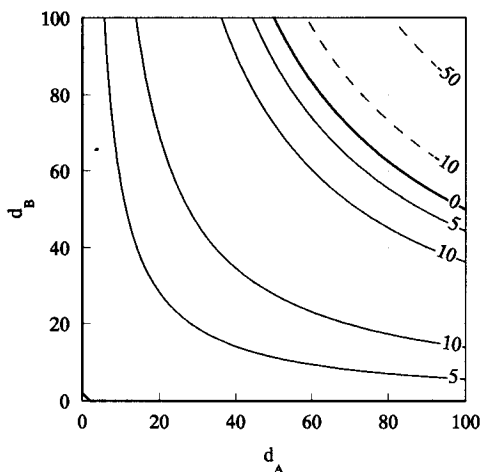


Fig. 11: Contour plot for the difference response surface shown in Fig. 10.

D_B or both have to be assumed to be infinite. This leads in the latter case to $I = 0$.

A further problem arises with non-monotonic dose-response curves. In this case there is more than one single-agent dose with the same effect. Hence, one can calculate different I values for one dose combination. Moreover, the interpretation of values of $I < 1$ as synergistic and of $I > 1$ as antagonistic relies on the assumption that the dose-response relations are monotonic. The application of the isobole approach to dose-response relations with non-monotonic dose-response curves requires a more detailed investigation which is to be described in a paper to follow.

However, a short discussion for the response surface of eq. (41), Fig. 4 and 5, will be given here.

The maximum effect of the single agents has a value of 0.4225 and is reached at a dose of 65. The effect of the response surface within a circle with its origin at $d_A = 65$, $d_B = 65$ and a radius of 65 is larger than 0.4225. Thus, in this dose range the value of I is equal to zero. The interaction function $I(d_A, d_B)$ is given by

$$I(d_A, d_B) = d_A / \{ (\alpha/2\beta) \pm [(\alpha/2\beta)^2 - (\alpha/\beta)(d_A + d_B) + (d_A^2 + d_B^2)]^{1/2} \} + d_B / \{ (\alpha/2\beta) \pm [(\alpha/2\beta)^2 - (\alpha/\beta)(d_A + d_B) + (d_A^2 + d_B^2)]^{1/2} \} \quad (47)$$

for

$$(d_A^2 + d_B^2 - 130(d_A + d_B) + 4225 \geq 0)$$

and

$$I(d_A, d_B) = 0$$

for

$$(d_A^2 + d_B^2 - 130(d_A + d_B) + 4225 < 0).$$

For dose combinations within the circle mentioned previously equation (47) is not defined due to negative values of the square root. In this case $I(d_A, d_B)$ is assumed to be equal to zero. At first glance, one can see that, due to the two \pm signs, there are four different I values for each dose combination. By means of the isobologram shown in Fig. 5 one can, however, easily choose the correct values of D_A and D_B for any dose combination. For the isobole with an effect of 0.4 all dose combinations for which both d_A and d_B are smaller than 50 require D_A and D_B equal to 50. All dose combinations for which d_A and/or d_B are larger than 80 require D_A and D_B equal to 80.

Even if this selection of the correct values of D_A and D_B would not be possible it would turn out that, according to eq. (47), all I values for dose combinations with $d_A + d_B < 65$ are smaller than 1 and all I values for dose combinations with $d_A + d_B > 65$ are larger than 1. This would indicate synergism in the low dose range and antagonism in the remaining range outside the region with $I(d_A, d_B) = 0$. The latter interpretation, however, is incorrect. This is due to the fact that the response curves decrease with increasing dose. In this case the interpretation of I has to be reversed.

The difference response surface (Fig. 12) yields the correct evaluation of interaction in the whole dose range. Note that the zero interaction response surface (Fig. 3) is positive only for $d_A + d_B < 130$. For larger values of $d_A + d_B$ the zero interaction response is thus assumed to be zero. The difference response surface and the corresponding contour plot show that the whole response surface is synergistic.

The difference response surfaces can also be used if there is no explicit expression for the zero interaction response surface. In this case implicit equations (see for example eqs. (7), (14), (19), (24), (29), (35)) have to be solved numerically. Current computer graphics software enables one very easily to display the zero interaction response surface or any difference response surface calculated in this way. An example is shown in Fig. 13.

5. Application

The methods described so far represent a variety of different procedures for evaluating a possible interaction between biologically active agents, which, however, are all based on the isobole equation (1) and which should thus, if applied correctly, yield identical results. Due to this variety they can satisfy very different requirements of the particular experimental data set. In this work the focus

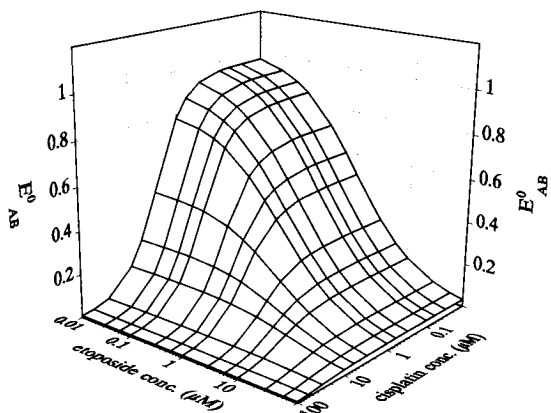


Fig. 13: Zero interaction response surface E_{AB}^0 for the combined cytotoxic effect of etoposide and cis-diammine-dichloroplatinum(II) on the human carcinoma cell line NCI-H226 in ACL-4 medium (effect: relative control absorbance; eq. (49); cisplatin: $\alpha = 2.75$, $\mu = 1.34$; etoposide: $\alpha = 1.02$, $\mu = 0.74$; experimental data after [12], Table 2).

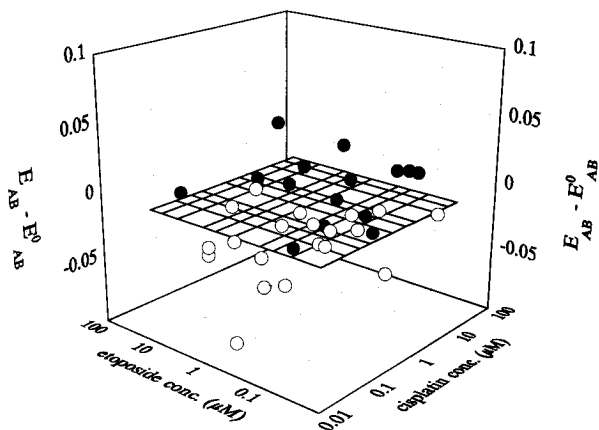


Fig. 14: Difference $E_{AB} - E_{AB}^0$ for the combined effect of etoposide and cis-diammine-dichloroplatinum(II) on the human carcinoma cell line NCI-H226 in ACL-4 medium (E_{AB} stands for the experimental data points and E_{AB}^0 for the expected effect for zero interaction calculated from the single-agent dose-response relations according to eq. (49); full circles indicate positive values and empty circles negative values of this difference; the values below the zero plane (empty circles) are shown in all cases even if they are covered by grid lines of the zero plane).

is on the description of new methods for the evaluation of a possible interaction between biologically active agents. Nevertheless, for one experimental example the application of one of the methods proposed is described in the following. Tsai et al. reported on the cytotoxic effect of etoposide and cis-diammine-dichloroplatinum(II) on human lung carcinoma cell lines presenting an impressive amount of experimental data [12]. They used a method for assessing the interaction between the agents used which is usually called "envelope additivity method" [13]. This is essentially a modified isobole method which is identical with the classical isobole approach for linear dose-response relations but leads to other results for other dose-response relations. A substantial number of experiments with different cell lines shows a marked synergism according to the classical isobole approach but no interaction according to the envelope additivity method. Berenbaum has analyzed this latter method in detail [1]. He has shown that this method suffers from various inconsistencies and that there is no need to apply it instead of the classical approach. In the following one of the data sets provided by Tsai et al. (Table 2 of their work) is used for which both methods yield no interaction for the 50% effect. Even though Tsai et al. claim to report on the first study which explores such a large proportion of the three-dimensional dose-effect surface, they display, in fact, only classical isobolograms for one effect level. In the following it is shown that applying one of the methods proposed in this paper enables one to evaluate the interaction in the complete dose range under study, that means for all effect levels, using the very same experimental data set. For the single agents a dose-response relation, eq. (48), was used which is also called logistic, which, however, is not identical with eq. (42), but bears resemblance to the median-effect relation, eq. (28).

$$E(d) = 1/[1 + (d/\alpha)^\mu] \quad (48)$$

The corresponding zero interaction response surface is given by the implicit equation

$$d_A/\{\alpha_A [(1-E_{AB}^0)/E_{AB}^0]^{1/\mu_A}\} + d_B/\{\alpha_B [(1-E_{AB}^0)/E_{AB}^0]^{1/\mu_B}\} = 1 \quad (49)$$

Assuming $\mu_A = \mu_B = \mu$ the simplified eq. (50) is obtained

$$E_{AB}^0 = 1/[1 + \{(d_A/\alpha_A) + (d_B/\alpha_B)\}^\mu] \quad (50)$$

In eqs. (48)–(50) α stands for the dose which produces a 50% effect (IC_{50}) and μ governs the slope of the dose-response relations. The α values were taken from the Tsai

paper and the μ values were determined by nonlinear regression of eq. (48) using the corresponding option of the STATGRAPHICS software package (version 3.0) [cisplatin: $\alpha = 2.75$, $\mu = 1.34$ ($r = 0.9917$); etoposide: $\alpha = 1.02$, $\mu = 0.74$ ($r = 0.9793$)]. Due to the different values of μ the simplified eq. (50) could not be used. Therefore, eq. (49) was solved by iteration for a great number of dose combinations. By means of current computer graphics software it is a simple task to display data obtained in this numerical way. In Fig. 13 the corresponding zero interaction response surface is shown. A closer examination of the experimental data shows that there is no substantial deviation from the zero interaction response surface in the whole dose range studied. This can be seen in Fig. 14. Here the differences between the individual experimental data points and the effects expected for zero interaction are shown. If all effects were indeed zero-interactive all points would lie in the zero plane shown. The deviations from the plane are rather small within a range of ± 0.06 for all but one data point. In other words, the combination of etoposide and cisplatin does not display any interaction in the dose range studied. Note, that this conclusion is far more comprehensive than the result obtained by Tsai et al. (no interaction for the 50% effect level) even though the very same data set was used.

This work is not concerned with the evaluation of statistical significance. The selection of the most appropriate statistical test is very dependent on the particular data set studied and on the method used for the evaluation of interaction. Very instructive examples are given in [2], pp. 69.

6. Conclusions

The combination of the isobole equation with dose-response relations of single-agents leads to relationships between single-agent doses or effects, the combined effect and the interaction index I . These relationships can be used to define zero interaction response surfaces, difference response surfaces and interaction functions. The zero interaction response surface represents a surface which shows zero interaction in the whole dose range. The difference response surface displays the difference between an actual response surface and the corresponding zero interaction response surface. The zero line in the corresponding contour plot indicates zero interaction.

The interaction function represents a generalization of the interaction index, which displays the dose dependence of this quantity. The contour plot of the interaction function shows lines of equal interaction and is thus called iso-interaction diagram. For non-monotonic dose-response relations one runs into difficulties if the index of interaction or the interaction function is to be calculated. These difficulties can be overcome by means of the difference response surface. Whereas the interaction index and the zero interaction response surface can also be calculated for a small number or even one dose combination, provided the single-agent dose-response relations are known, the generation of difference response surfaces and interaction functions requires an experimental design appropriate for surface modeling of response data or interaction indices. In any case response surface modeling and computer graphics enable one to perform the evaluation of a possible interaction in the whole dose range under study from a relatively small number of dose combinations. Finally, it should be noted that interaction indices, zero interaction response surfaces, difference response surfaces and interaction functions should lead to identical results provided the modeling procedure is done appropriately.

Upon finalization of this work we became aware of two related papers [7, 9]. In [7] our concept of a difference response surface is used without, however, applying this term. Unfortunately, zero interaction effects are calculated simply by adding the effects of single agents. As shown by Berenbaum [1] and in this paper, eq. (4), this is only correct for linear dose-response relationships [14]. In [9] a universal response surface approach is suggested. In this work the isobole equation is also combined with a particular dose-response relation (the median-effect equation (28)). This leads for the case of zero interaction to correct equations identical with eqs. (29) and (31) of this work. The authors then arrive at the final equation by adding an interaction term which contains an interaction parameter α . The parameter α is assumed to be constant over the whole dose range. This is at variance with the well-known fact that extent and even type of interaction may be dose dependent. Hence, the author's claim that their universal response surface method is consistent with the traditional isobologram approach is not correct [15].

7. References

- [1] Berenbaum, M. C., *Pharmacol. Rev.* **41**, 93 (1989) — [2] Unkelbach, H. D., Wolf, T., *Qualitative Dosis-Wirkungs-Analysen, Einzelsubstanzen und Kombinationen*, Gustav-Fischer-Verlag, Stuttgart (1985) — [3] Unkelbach, H.-D., Pösch, G., *Arzneim.-Forsch./Drug Res.* **38** (I), 1 (1988) — [4] Berenbaum, J., *Theor. Biol.* **114**, 413 (1985) — [5] Gennings, C., Carter, W. H. Jr., Harris, L. W., Carchman, R. A., Campbell, E. D., Boyle, R. M., Talbot, B. G., Solana, R. P., *Fundam. Appl. Toxicol.* **14**, 235 (1990) — [6] Sühnel, J., *Antiviral Res.* **13**, 23 (1990) — [7] Prichard, M. N., Shipman, C. Jr., *Antiviral Res.* **14**, 23 (1990) — [8] Baumgart, J., Schlott, B., Sühnel, J., Vater, W., Schulze, W., Behnke, D., *J. Cancer Res. Clin. Oncol.* **117**, 239 (1991) — [9] Greco, W. R., Park, H. S., Rustum, Y. M., *Cancer Res.* **50**, 5318 (1990) — [10] Laffort, P., in: *Perception of Complex Smells and Tastes*, D. G. Laing, W. S. Cain, R. McBride, B. W. Ache, (eds.), pp. 205, Academic Press, Sydney (1989) — [11] Bois, F. Y., Vasseur, P., *J. Natl. Cancer Inst.* **74**, 729 (1985) — [12] Tsai, C.-M., Gazdar, A. F., Venzon, D. J., Steinberg, S. M., Dederick, R. L., Mulshine, J. L., Kramer, B. S., *Cancer Res.* **49**, 2390 (1989) — [13] Steel, G. G., Peckham, M. J., *Int. J. Radiat. Oncol. Biol. Phys.* **5**, 85 (1979) — [14] Sühnel, J., *Antiviral Res.* **17**, 91 (1992) — [15] Sühnel, J., *Cancer Res.*, ~~in press~~ **52**, 4558 (1992)

Acknowledgements

This work was supported by a grant of the Deutsche Forschungsgemeinschaft. Stimulating hints of one of the referees and stylistic improvements by Andrew Pearson are gratefully acknowledged. The computer program for solving the implicit equations was written by Gordon Bank.

Correspondence: Dr. J. Sühnel, Institute of Molecular Biotechnology, Beutenbergstr. 11, O-6900 Jena (Fed. Rep. of Germany)