



Parallel Dose–Response Curves in Combination Experiments

JÜRGEN SÜHNEL

Institut für Molekulare Biotechnologie,
Postfach 100813,
D-07708 Jena, Germany

E-mail: jsuehnel@imb-jena.de

A possible experimental design for combination experiments is to compare the dose–response curve of a single agent with the corresponding curve of the same agent using either a fixed amount of a second one or a fixed dose ratio. No interaction is then often defined by a parallel shift of these curves. We have performed a systematic study for various types of dose–response relations both for the dose-additivity (Loewe additivity) and for the independence (Bliss independence) criteria for defining zero interaction. Parallelism between dose–response curves of a single agent and those of the same agent in the presence of a fixed amount of another one is found for the Loewe-additivity criterion for linear dose–response relations. For nonlinear relations, one has to differentiate between effect parallelism (parallel shift on the effect scale) and dose parallelism (parallel shift on the dose scale). In the case of Loewe additivity, zero-interaction dose parallelism is found for power, Weibull, median-effect and logistic dose–response relations, given that special parameter relationships are fulfilled. The mechanistic model of competitive interaction exhibits dose parallelism but not effect parallelism for Loewe additivity. Bliss independence and Loewe additivity lead to identical results for exponential dose–response curves. This is the only case for which dose parallelism was found for Bliss independence. Parallelism between single-agent dose–response relations and Loewe additivity mixture relations is found for examples with a fixed dose–ratio design. However, this is again not a general property of the design adopted but holds only if special conditions are fulfilled. The comparison of combination dose–response curves with single-agent relations has to be performed taking into account both potency and shape parameters. The results of this analysis lead to the conclusion that parallelism between zero interaction combination and single-agent dose–response relations is found only for special cases and cannot be used as a general criterion for defining zero-interaction in combined-action assessment even if the correct potency shift is taken into account.

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1. INTRODUCTION

Studies of the combined action of biologically active agents, such as drugs, carcinogens, environmental pollutants, radiation, odorants, taste stimuli, etc. become

increasingly important in many branches of biomedical research. This is, for example, confirmed by the fact that recent search (March 5, 1997) for the character pattern 'synerg*' (synergism, synergistic) using the Internet-based PubMed Retrieval system (<http://www4.ncbi.nlm.nih.gov/PubMed/>) resulted in 50,850 hits. Unfortunately, there is widespread disagreement over terminology, definitions and models for the evaluation of interaction (Berenbaum, 1989; Greco *et al.*, 1992, 1995; Gebhart, 1992; Miaskowski and Levine, 1992b; Suzuki, 1994). Hence, there is an urgent need for standardization in assessing a possible interaction in combination experiments. The usual approach is to calculate from the effects of single agents what is expected for the combination effect in the case of 'no interaction,' whatever this means. If the effect observed is larger or smaller than expected one speaks of an 'interaction.'

There are various models for calculating the expected effects. Recently, the dose-additivity model has been called the 'gold standard' (Gebhart, 1992). It is beyond the scope of this work to discuss meaning and limits of the various models in detail. This was done by others and by us in other papers (Berenbaum, 1989; Sühnel, 1990, 1992a, 1992b; Greco *et al.*, 1992, 1995). Here, we can simply state that, taking into account all arguments, we are led to the conclusion that, this model is indeed the most appropriate empirical criterion for evaluating combination experiments. Probably the most convincing argument is that it is the only model which never yields an interaction for the sham combination of an agent with itself. On the other hand, we should be aware of the fact that at least one further model, the so-called independence criterion, deserves attention. Both models are recommended by the so-called Saariselkä agreement (Greco *et al.*, 1992). The terminology proposed by these authors is Loewe additivity for the dose-additivity criterion and Bliss independence for the independence criterion. The cases for which the combination effects deviate from the zero-interaction effect are then called Loewe/Bliss synergism/antagonism. This terminology avoids the difficulties which usually arise when the terms synergism or antagonism are used without stating explicitly according to which criterion the evaluation of the combination experiment was performed.

A possible procedure in combined-action assessment is to compare the single-agent dose-response curve with the dose-response curve of the same agent in combination with a fixed dose of another drug. The statistical significance of the comparison of the two dose-response curves is then often tested using the two-factor repeated-measures analysis of variance (ANOVA) method. A few authors seem to assume that a significant value of a so-called interaction term, which is a possible result of the ANOVA analysis, means deviation from dose additivity, and that a parallelism of the two dose-response relations defines no interaction (Harvey and Klaassen, 1983; Caplan and Su, 1986; Berger *et al.*, 1987; Loomis *et al.*, 1988; Brennan and Jastreboff, 1989; Katahira *et al.*, 1989; Kopia *et al.*, 1989; Sato *et al.*, 1989; Shaikh *et al.*, 1989; Woodward *et al.*, 1989; Miaskowski *et al.*, 1990, 1992a; Oksenberg *et al.*, 1990; Thompson and Epstein, 1991; Sutters *et*

al., 1992; Mao *et al.*, 1992). Caudle and Williams (1993) criticized this approach and showed for one example that it is not always correct to assume that parallel dose–response curves indicate no interaction according to dose additivity, using as an example, a simple pharmacological receptor model.

The analysis of combination experiments by comparing single-agent dose–response relations with the relation of the same agent in the presence of a fixed amount of a second one is also especially recommended in a recent book on combined-action assessment (Pösch, 1993) and in other papers of the same author, see, for example, Pösch *et al.*, (1990). It is assumed that both dose–response curves exhibit a parallel shift on a linear-dose scale for the case of dose additivity.

A parallel line assay was also adopted by Tallarida *et al.* (1989), who used probit-type dose–response curves. Contrary to the work cited above, they claimed that for single-agent dose–response curves of equal slope and using a fixed dose ratio of the two agents combined, both additive and nonadditive mixture relations are parallel to the single-agent relations. This means that deviation of additivity is not indicated by a deviation from parallelism but by the extent of the shift compared with the parallel additivity line.

Chou and Talalay (1984), for their case of mutually exclusive inhibitors, (which is identical with the Loewe-additivity criterion), also found parallel additivity lines for a fixed dose ratio given that the single-agent relations are parallel.

To address this problem in a more comprehensive and general way, analytical expressions for the combination effects in terms of doses have to be known. This is a problem for the dose-additivity model, because it is defined in terms of doses alone. We have recently combined response-surface modeling techniques with the dose-additivity (Loewe-additivity) criterion (Sühnel, 1990, 1992a, 1992b, 1993, 1996). This led to mathematical expressions for response surfaces which define zero interaction throughout the complete dose range and are therefore called zero-interaction response surfaces. Single-agent dose–response relations and dose–response curves of an agent in the presence of a fixed amount of a second one, or with a fixed dose ratio, represent cross sections through the corresponding response surface. In this way, the question of whether a combination is characterized by parallel dose–response curves or not can be addressed in a much more comprehensive manner, as by Caudle and Williams (1993) or Tallarida *et al.* (1989). (Figures 3 and 4 of Caudle and Williams (1993) show an isobologram and a response surface with linear isoboles and logarithmic dose scales, which are claimed to represent dose additivity; this is an error. Linear isoboles require linear dose scales. For logarithmic dose scales, additive isoboles are curved.)

The objective of this work was to present a general answer to the question for which cases parallel dose–response curves have to be expected for the case of zero interaction.

2. ZERO INTERACTION RESPONSE SURFACES

As already noted, there are different criteria for defining zero interaction in combination experiments. We follow the recommendations of the Saariselka agreement (Greco *et al.*, 1992) and take into account Bliss independence and Loewe additivity. The independence criterion (Bliss independence) was originally derived from probability theory. In this case the expected effect for a combination of two agents can be calculated from the single-agent effects $E_A(d_A)$, $E_B(d_B)$ by

$$E_{AB}^{BI} = E_A + E_B - E_A E_B \quad (1)$$

where, for the sake of brevity, the dependence of the effects on the doses d_A and d_B of agents A and B is not written out. Note that equation (1) can only be applied to fractional effects $0 < E < 1$. In this case the effect can simply be replaced by the fractional survival, S , using $E = 1 - S$. The dose-additivity criterion (Loewe additivity) defines zero interaction by equation (2):

$$(d_A/D_A) + (d_B/D_B) = I \quad (2)$$

where the interaction index $I = 1$. $I < 1$ represents synergism and $I > 1$ antagonism. In equation (2), d_A , d_B are the combination doses, and D_A and D_B stand for the doses of agents A and B which produce the same magnitude of effect when used alone.

The usual procedure of applying the Loewe-additivity criterion is either to calculate the index of interaction I according to equation (2) or to plot an isobologram. An isobologram is a two-dimensional graph with the doses of agents A and B as coordinate axes, in which one or several lines, the isoboles, are shown connecting different dose combinations which all produce the same magnitude of effect. This means that the Loewe-additivity isoboles ($I = 1$) are straight lines in an isobologram with linear dose scales touching the dose axes at the single-agent doses D_A and D_B . To the best of our knowledge an isobologram, and thus indirectly, equation (2), was first used in Fraser (1870–1871). Later, this method was especially advocated by Loewe (1953). Although widely used, the theoretical basis of this equation remained unclear for a long time, and even now its application is subject to discussion. A detailed presentation of the arguments leading to equation (2) starting out from empirical monotonic dose–response relations was given by Berenbaum (1989). He arrived at the conclusion that equation (2) is valid irrespective of the shapes of the dose–response curves and the mechanism of action. We have recently reinforced his arguments and shown that the Loewe-additivity method can be applied to nonmonotonic dose–response relations as well (Sühnel, 1992b). For the nonmonotonic case, however, the usual interpretation of $I < 1$ as synergism and $I > 1$ as antagonism has to be reversed in passing from the increasing to the decreasing part of the nonmonotonic dose–response relation.

It is important to note that Chou and Talalay (1984), using mass-action considerations, also derived equation (2) for their case of mutually exclusive inhibitors. Further, one can easily show that the classical model of competitive interaction proposed by Ariëns *et al.* (1956) fulfills equation (2) at least in an effect range up to the lower maximum effect (Sühnel, 1992b).

The calculation of zero-interaction response surfaces for Bliss independence is straightforward (equation (1)). It simply requires the insertion of the single-agent dose–response relations into the corresponding equation. For the Loewe additivity criterion, the approach is more involved, because the definition is given in terms of doses and not effects, as already noted. However, in this case the single-agent dose–response relations can be recast after the dose and then inserted into equation (2). With

$$E(d_A, d_B) = E(D_A) = E(D_B) \quad (3)$$

this also leads to expressions for the expected combination effect. We have derived expressions for various widely used empirical dose–response relations (Sühnel, 1992a, 1996).

The expressions for a few empirical dose–response relations are given as examples. Empirical means here that simply information on doses and effects is required. The quantity $E_{AB}^{LA}(d_A, d_B)$ defines the surface of zero interaction (Loewe-additivity surface). $E_{AB}^{LA}(d_A, d_B)$ is abbreviated by E_{AB}^{LA} for the sake of brevity and α , β , μ and σ are parameters. The equations for most of the dose–response relations were already reported in recent papers (Sühnel, 1992a, 1996). The equations for the logistic relation are new.

We do not consider a possible mechanistic background as in the case of the median-effect equation, which was derived from mass-action considerations, and we do not discuss the meaning of the parameters such as α in the same equation, which corresponds to the ED_{50} value (Chou and Talalay, 1984). It should be noted, however, that the median-effect equation is identical to the Hill equation, which is widely used in pharmacology (Hill, 1913). In the following, first the single-agent dose–response relation is given. Then the general expression for the Loewe-additivity response surface for the same type of single-agent dose–response relation with possibly different parameters is presented. This is usually an implicit equation. If we assume that special parameter relationships hold, for example $\mu_A = \mu_B = \mu$, then, in all cases discussed here, the general expression simplifies to an explicit equation. Even though it is not used here, Loewe-additivity surfaces can also be derived for combinations of single-agent dose–response relations of a different mathematical type.

Power-function dose–response relation (which includes the linear relation):

$$E(d) = (d/\alpha)^\mu \quad (4)$$

$$\{d_A/[\alpha_A(E_{AB}^{LA})^{1/\mu_A}]\} + \{d_B/[\alpha_B(E_{AB}^{LA})^{1/\mu_B}]\} = 1 \quad (5)$$

$$E_{AB}^{LA} = [(d_A/\alpha_A) + (d_B/\alpha_B)]^\mu \quad (\mu_A = \mu_B = \mu). \quad (6)$$

Median-effect relation:

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

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

$$E_{AB}^{LA} = [(d_A/\alpha_A) + (d_B/\alpha_B)]^\mu / \{1 + [(d_A/\alpha_A) + (d_B/\alpha_B)]^\mu\} \quad (\mu_A = \mu_B = \mu). \quad (9)$$

Weibull relation:

$$E(d) = 1 - \exp[-(\alpha d)^\mu]; \quad S(d) = \exp[-(\alpha d)^\mu], \quad (10)$$

where S is the survival fraction, which is related to the effect E by $S = 1 - E$. Note that S is always restricted to the range $0 < S < 1$.

$$\alpha_A d_A / (-\ln S_{AB}^{LA})^{1/\mu_A} + \alpha_B d_B / (-\ln S_{AB}^{LA})^{1/\mu_B} = 1 \quad (11)$$

$$S_{AB}^{LA} = \exp[-(\alpha_A d_A + \alpha_B d_B)^\mu] \quad (\mu_A = \mu_B = \mu). \quad (12)$$

Logistic relation:

$$E(d) = 1 - \{1/[1 + (d/\sigma)^\mu]\} \quad (13)$$

$$\{(d_A/\sigma_A)/[E_{AB}^{LA}/(1 - E_{AB}^{LA})]^{1/\mu_A}\} + \{(d_B/\sigma_B)/[E_{AB}^{LA}/(1 - E_{AB}^{LA})]^{1/\mu_B}\} = 1 \quad (14)$$

$$E_{AB}^{LA} = 1/\{1 + [(d_A/\sigma_A) + (d_B/\sigma_B)]^\mu\} \quad (\mu_A = \mu_B = \mu). \quad (15)$$

Linear-logarithmic relation:

$$E(d) = \alpha \log d + \beta \quad (16)$$

$$\{d_A/10^{[(E_{AB}^{LA}-\beta_A)/\alpha_A]}\} + \{d_B/10^{[(E_{AB}^{LA}-\beta_B)/\alpha_B]}\} = 1. \quad (17)$$

For $\alpha_A = \alpha_B = \alpha$ and $\beta_A = \beta_B = \beta$ the equation simplifies to

$$E_{AB}^{LA} = \alpha \log(d_A + d_B) + \beta \quad (18)$$

and for $\alpha_A = \alpha_B = \alpha$ and $\beta_A \neq \beta_B$ the following equation is obtained

$$E_{AB}^{LA} = \alpha \log[10^{(\beta_A/\alpha)} d_A + 10^{(\beta_B/\alpha)} d_B]. \quad (19)$$

The possible parameter ranges can easily be derived from the single-agent dose-response relations. The mathematical expressions for the Loewe-additivity

response surfaces represent often implicit relations which have to be solved by iteration. Explicit expressions are obtained for special parameter relationships.

In three seminal papers, Ariëns *et al.* (1956) proposed several mechanistic models for the interaction of biologically active agents. Based on mass-action considerations, it was assumed that the concentration dependence of effect E of a single agent can be described by equation (20). Note that the concentrations c used in the following can be simply replaced by doses d .

$$E(c) = E_{\max}c/[K + c] \quad (20)$$

where 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 and E_{\max} is given by

$$E_{\max} = \gamma c_R, \quad (21)$$

where c_R is the total receptor concentration and γ is an intrinsic activity.

Assuming that two agents A and B interact with one and the same receptor system, Ariëns *et al.* (1956) arrived at the following equations:

$$E_{AB}(c_A, c_B) = \alpha c_{RA} + \beta c_{RB}, \quad (22)$$

$$E_{AB}(c_A, c_B) = [E_{\max}^A K_{BCA} + E_{\max}^B K_{ACB}]/[K_{ACB} + K_{BCA} + K_A K_B]. \quad (23)$$

In equation (22) c_{RA} , c_{RB} are the concentrations of the receptor occupied by agents A or B, and α and β are intrinsic activities. Note that equation (23) was derived under the assumption that c_A and c_B are large compared with c_R . In this case it can be assumed that the initial and equilibrium concentrations of A and B are equal. The type of action described by equation (23) is usually called competitive interaction. Equation (23) is identical to the expression used by Caudle and Williams (1993).

Contrary to the empirical relations described above both the single-agent dose–response relation (20) and the relation for the combination effect (23) were derived from mechanistic considerations. Nevertheless, for the moment we can forget about the mechanistic background and look at equation (20) from a purely empirical point of view. Adopting the same procedure as for the other dose–response relations, equation (20) can be combined with equation (2). The result obtained in this way for $E_{AB}^{LA}(d_A, d_B)$, equation (9) with $\mu = 1$, is identical to the expression derived by the Ariëns Equation (23). In other words, the relationship derived from mechanistic considerations is identical to the result obtained independently adopting the empirical Loewe-additivity model. This is a more general confirmation of the statement of Caudle and Williams (1993) that their equation fulfils the dose-additivity criterion. On the other hand—and this is more important—this is a mechanistic explanation of Loewe-additivity. Note that this

relationship between empirical and mechanistic models holds only for $\mu = 1$ in equation (9). Further, it should be noted that, using the median-effect equation, Chou and Talalay (1984) derived equation (2) for the case of mutually exclusive agents. This means that they also related their model to the empirical equation (2). Work on a detailed comparison of the empirical and mechanistic models is in progress.

The zero-interaction response surfaces contain all information on the dose-dependence of combination effects including the single-agent dose-response relations for agents A and B [$E_{AB}^0(d_A, d_B = 0)$] and B [$E_{AB}^0(d_A = 0, d_B)$], the dose-response relations of an agent, say A, in the presence of a fixed amount of agent B [$E_{AB}^0(d_A, d_B = \text{const.})$] or the dose-response relations for a simultaneous variation of d_A and d_B keeping the dose ratio fixed [$E_{AB}^0(d_A/d_B = \text{const.})$] and it is, therefore, appropriate to address the question of parallel dose-response curves (E_{AB}^0 —zero interaction response surface according to Loewe-additivity or Bliss independence).

3. PARALLEL DOSE-RESPONSE CURVES

When comparing single-agent with combination dose-response curves, one has to consider all parameters describing the corresponding relations. In many cases, one can differentiate between parameters defining the position of the dose-response curve on the dose axis and others governing the shape. For example, in the logistic relation (eq. (13)) the parameter σ corresponds to the dose required for a 50% effect, and can therefore be viewed as a potency parameter, whereas μ determines the steepness of the relation. For the combination of an agent with a fixed dose of a second one, the shift of the combination curve compared with the single-agent curve is defined by the combinations $E_{AB}(d_A = 0, d_B = 0)$ and $E_{AB}(d_A = 0, d_B = \text{const.})$. This means that if these dose combinations are part of the dose-response curves studied, the extent of the shift on the dose scale is automatically defined by the dose combinations given above and it is sufficient to analyze the shape. If these dose combinations are not taken into account—for example—when using logarithmic dose scales, one has in any case to analyze both the shape and shift of the dose-response relations.

Except for linear dose-response curves, we have further to differentiate between curves shifted on the effect scale (effect parallelism) and on the dose scale (dose parallelism). Both models were used as criteria for the assessment of no interaction: effect parallelism (Mao *et al.*, 1992; Miaskowski *et al.*, 1992a and other papers cited by these authors, and further papers cited by Caudle and Williams, 1993), dose parallelism (Pöch *et al.*, 1990; Pöch, 1993). The following analysis refers directly to the equations presented without considering possible transformations. It is primarily concerned with the comparison of single-agent dose-response relations with the dose-response relation of the same agent in the presence of a fixed amount of a second agent. Finally, the possible parallelism

between single-agent curves and combination relations adopting a fixed-dose ratio is discussed.

Effect-parallelism:

The dose-response curve of A alone and of A in the presence of a fixed amount of B are parallel on the effect scale if the relation

$$E_{AB}(d_A, d_B = \text{const.}) - E_A(d_A) = K_E \quad (24)$$

is fulfilled, where K_E stands for a dose-independent constant effect. For the explicit equations (6), (9), (12), (15) and (18) and the Ariëns equation (23) one can easily see that for the Loewe-additivity response surfaces this criterion is not fulfilled except for the linear dose-response relation (equation (4); $\mu = 1$). Note, that equation (18) is not linear for varying d_A and constant d_B , even though the single-agent relation (16) represents a straight line on a logarithmic dose scale. The analysis of the implicit equations (5), (8), (11), (14) and (17) is more involved but the result is the same.

The Bliss independence criterion cannot be applied to the power function and linear-logarithmic dose-response relations, because in this case the effect cannot be written as a fractional effect. For the other dose-response curves, we do not find an example for which equation (24) is fulfilled. This can be shown in a general way. For Bliss independence, K_E is given by equation (25).

$$K_E = E_B(d_B = \text{const.})[1 - E_A(d_A)] \quad (25)$$

It is obvious that K_E can be constant only if E_A is dose independent.

Dose-parallelism:

The criterion for parallel dose-response curves on the dose level for the same design as used above is

$$d_A^a - d_A^c = K_D \quad E_A(d_A^a) = E_{AB}(d_A^c, d_B = \text{const.}) \quad (26)$$

In equation (26) d_A^a and d_A^c are the doses of agent A alone and in combination, and K_D is the constant dose shift. We have already mentioned that for linear relations dose parallelism implies effect parallelism and vice versa. Therefore, the question here is whether or not there are nonlinear relations which do not show effect parallelism but nevertheless do show dose parallelism. Let us first discuss the Loewe-additivity response surfaces for the empirical equations (4), (7), (10) and (13). The analysis shows that equation (26) is not fulfilled for the general case, but holds for $\mu_A = \mu_B = \mu$ in all cases. Of course, this includes the linear relation as well. The quantity K_D is given by $(\alpha_A/\alpha_B)d_B$ for the power function and for the median effect relation, by $(\alpha_B/\alpha_A)d_B$ for the Weibull relation and by $(\sigma_A/\sigma_B)d_B$ for the logistic function.

For the linear-logarithmic relation (16) we had already noted that the Loewe-additivity relation for varying d_A and fixed d_B is not linear and parallel to the single-agent relation.

Equation (20), which is the basis for the Ariëns equation (23), is identical to the median-effect equation (7) with $E_{\max} = 1$ and $\mu = 1$. This means that the results obtained for the median-effect equation can be applied to the Ariëns equation as well. In other words, even though equation (23) does not display effect parallelism, as pointed out correctly by Caudle and Williams (1993) and as shown above, it fulfils equation (26) and has thus the property of dose parallelism for $E_{\max}^A = E_{\max}^B$.

As already noted, the Bliss independence criterion cannot be applied to the power function relation (4) and to the linear-logarithmic equation (16). Bliss independence combination effects derived from equations (7), (10) and (13) do not fulfil equation (26) in general. However, there is a special case. It is well known that Bliss independence and Loewe-additivity criteria yield identical results for exponential dose-response curves, equation (10) with $\mu = 1$. We have found for Loewe-additivity that this dose-response relation type shows dose parallelism. Therefore, this holds for Bliss independence as well.

The Weibull relation with $\mu = 1$ can easily be linearized. This linearized equation shows effect and dose parallelism for both Loewe-additivity and Bliss independence, because in this case both criteria lead to identical results.

Figure 1 illustrates the results described for the logistic dose-response relation (equation (13)) for a varying dose of A and a fixed dose of B. Even though the relation is not linear there is a constant dose shift to the left by a dose of $(\sigma_A/\sigma_B)d_B = (40/60)30 = 20$ for $\mu_A = \mu_B = \mu$. On the other hand, for different values of μ there is no dose parallelism. The parallelism found for $\mu_A = \mu_B = \mu$ would disappear for a logarithmic dose scale.

A more simple situation occurs if one agent does not exhibit an effect when used alone but affects the response of the other agent in combination. In this case the definition of 'interaction' is very easy, because any effect of an agent in combination which does not show an effect, when used alone, can only be due to an 'interaction'. For a linear dose scale, this case always implies nonparallelism. For $d_A = 0$, $d_B = \text{const.}$ the difference is zero because B has no effect alone. If B influences the effect of A, then the difference is nonzero for any other dose of A.

The logistic dose-response relation is used by the Pöch group (Pöch *et al.*, 1990; Pöch, 1993), who claimed that the Loewe-additivity dose-response curve of an agent A in the presence of a fixed amount of B exhibits dose parallelism to the single-agent relation for A. According to our results this is correct for $\mu_A = \mu_B = \mu$. but not for $\mu_A \neq \mu_B$. For the combination of a varying dose of A with a fixed dose of B the dose-response relation of B is often not known. In this case one cannot be sure that the condition $\mu_A = \mu_B = \mu$ is indeed fulfilled and this can lead to wrong results. Therefore, it is recommended to determine

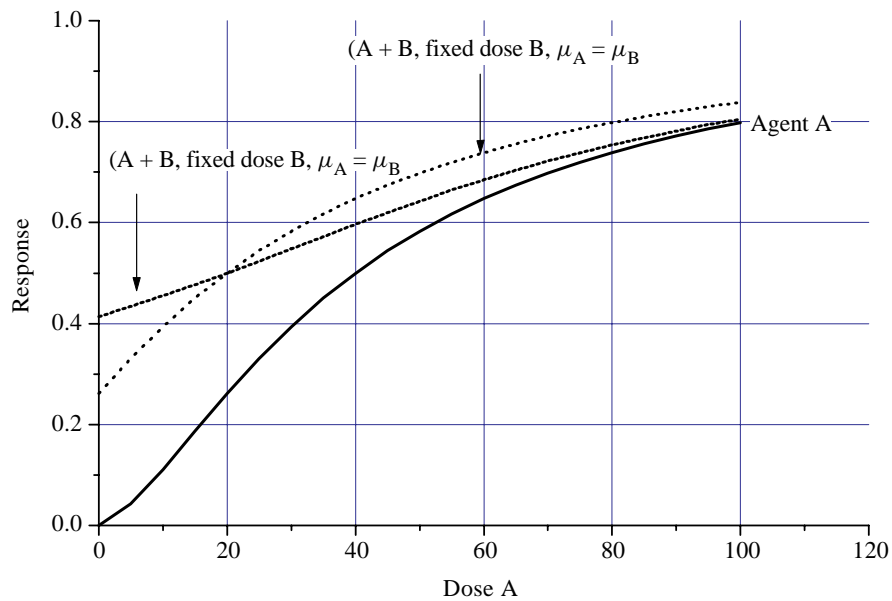


Figure 1. Logistic dose–response curves according to equations (13)–(15) for a single agent A (solid line) and for a combination of A with a fixed dose of agent B for the parameter relations $\mu_A = \mu_B = \mu$ (dotted line) and $\mu_A \neq \mu_B$ (dashed line). Equation (14) was solved using our program COMBITOOL, which will be released soon. Parameters: $\alpha_A = 40$; $\alpha_B = 60$; $d_B = 30$; $\mu_A = \mu_B = 1.5$; $\mu_A = 1.5$, $\mu_B = 0.5$.

the dose–response relations for both single agents. Then, the additivity relation for a varying dose of A and a fixed dose of B can be calculated. This curve may be parallel ($\mu_A = \mu_B = \mu$) or nonparallel ($\mu_A \neq \mu_B$) to the single-agent curve. A comparison of the experimental combination data with the calculated additivity curve leads to the final conclusion on additivity or nonadditivity of the combination data.

It has to be pointed out here that there is not yet a conclusion regarding whether the Loewe-additivity criterion can be applied to any combinations or only to agent combination with a similar action, whatever this means. In terms of empirical dose–response curves, it is often assumed that similar action means $\mu_A = \mu_B = \mu$ for the logistic equation, for example. However, as described above, Berenbaum (1989) provided good arguments that the Loewe-additivity criterion can be applied to any types of monotonic dose–response relations.

So far we have analyzed the parallelism of single-agent dose–response curves and of related curves of the same agent in the presence of a fixed amount of a second agent. This means that in this case the dose ratio between the two agents varies. There are other approaches in which single-agent dose–response relations are compared with dose–response relations for which the doses of both agents vary but their ratio is kept fixed (Chou and Talalay, 1984; Tallarida *et al.*, 1989). Tallarida *et al.* (1989) used the probit transformation to linearize dose–response relations. This corresponds to equation (16). Starting out with

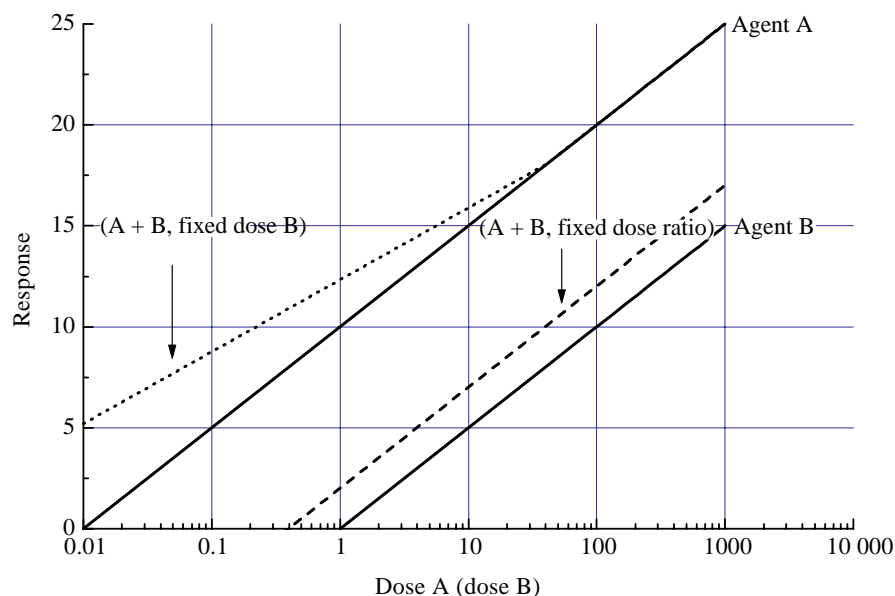


Figure 2. Linear-logarithmic dose–response curves according to equations (16)–(19) and (27) for the single agents A and B (solid lines) and for combinations of A and B with either a fixed dose of B (dotted line) or a fixed dose ratio (dashed line). Parameters: $\alpha_A = \alpha_B = 5$; $\beta_A = 10$; $\beta_B = 0$; fixed dose B: $d_B = 5$; fixed dose ratio: $K = d_B/d_A = 5$.

single-agent dose–response relations of equal slope ($\alpha_A = \alpha_B = \alpha$), it is claimed that both the relations indicating dose additivity and the real mixture are linear and parallel, and thus, only the extent of the shift is essential for the decision of whether the combination is additive. The general expression for the Loewe-additivity surface is given by equation (17). Simplified expressions for special parameter relationships are given in equations (18) and (19). Tallarida *et al.* (1989) adopted $\alpha_A = \alpha_B = \alpha$ and $\beta_A \neq \beta_B$ (equation (19)). One can easily see that for a variation of d_A and a fixed d_B the dose–response relation is not linear, and therefore also not parallel to the relation for agent A alone (Fig. 2). This is especially interesting because in this case the shape parameter α , which is analogous to μ for the logistic equation, is equal, nevertheless, the dose–response relations are not parallel. On the other hand, for a fixed dose ratio $d_B = K d_A$ (K -constant) the equation becomes

$$E_{AB}^{LA} = \alpha \log d_A + \log[10^{(\beta_A/\alpha)} + K 10^{(\beta_B/\alpha)}]. \quad (27)$$

This equation is indeed linear and parallel to both single-agent dose–response relations. Note that equation (27) provides an analytical expression for the expected shift of the additivity line compared with the dose–response relation of agent A alone. If the real mixture equation is also linear and parallel, then deviation from Loewe-additivity is indicated by a shift relative to the dose-additivity relation.

This would represent a case for which an interactive mixture dose–response relation is, nevertheless, parallel to the single-agent and to the Loewe-additivity relations. However, the real mixture equation can also be nonlinear or linear but not parallel. If the two single-agent relations are not parallel ($\alpha_A \neq \alpha_B$) then, of course, the Loewe-additivity relation is not parallel to any of the single-agent relations. It can easily be calculated by means of equation (17).

Chou and Talalay (1984) used the following linearized version of the median-effect equation (28).

$$\log[(E(d))^{-1} - 1]^{-1} = \mu \log(d/\alpha). \quad (28)$$

For two single-agent dose–response relations with equal slope the same linearization procedure can be applied to the equation describing the corresponding zero-interaction response surface according to the Loewe-additivity criterion for ($\mu_A = \mu_B = \mu$, equation 9). It leads to

$$\log[(E_{AB}^{LA}(d_A, d_B))^{-1} - 1]^{-1} = \mu \log[(d_A/\alpha_A) + (d_B/\alpha_B)]. \quad (29)$$

It is immediately obvious that the relation is not linear for varying d_A and fixed d_B . It is linear and parallel to the single-agent relations, however, for a fixed dose ratio when plotting the left-hand expression versus $\log d_A$ (Fig. 3).

This analysis has shown that for fixed dose ratios there are further examples of parallel dose–response relations in combination experiments. Even though it is not very likely, that nonadditive mixtures may have dose–response relations which are also linear and parallel to the single-agent and additive relations but are shifted compared with the latter ones.

The general conclusion of this analysis is that parallelism between dose–response curves of a single agent and the same agent in the presence of a fixed amount of another one indicates zero interaction according to the Loewe-additivity (dose-additivity) criterion for linear dose–response curves if the potency shift is correctly taken into account. For nonlinear relations one has to differentiate between effect parallelism and dose parallelism. In the Loewe-additivity case dose parallelism is found for the power, Weibull, median-effect and logistic dose–response relations given that special parameter relationships are fulfilled. The mechanistic model of competitive interaction exhibits dose parallelism but not effect parallelism for Loewe-additivity. The Bliss independence criterion cannot be applied to dose–response curves of the power function type because they do not approach maximum or minimum values. The only cases for which combination effects calculated according to the Bliss independence criterion are found are if this criterion and Loewe-additivity give identical results. This means that parallelism of the dose–response curves of a single agent and of the same agent in the presence of a fixed amount of another agent cannot be used as a general criterion for defining zero interaction in combination experiments. On

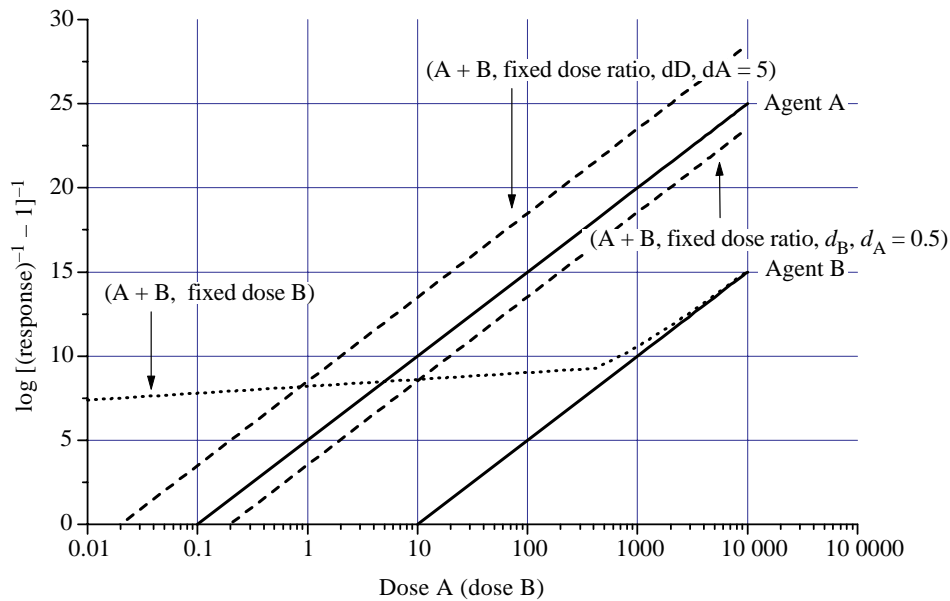


Figure 3. Transformed median-effect dose–response curves according to equations (28) and (29) for the single agents A and B (solid lines) and for combinations of A and B with either a fixed dose of B (dotted line) or a fixed dose ratio (dashed line). Parameters: $\mu_A = \mu_B = \mu = 5$; $\alpha_A = 10$; $\alpha_B = 0.1$; fixed dose: $d_B = 3$; fixed dose ratio: $K = d_B/d_A = 5$ and 0.5 .

the other hand, there are a few examples where even for nonlinear dose–response curve dose parallelism is found.

For combination studies in which the dose ratio between the combined agents is kept fixed, there are a few further cases for which the Loewe-additivity relation may be parallel to the single-agent relations. However, this is not a general property of the design adopted, but is correct only if certain conditions are fulfilled.

These results have the following implications.

- The comparison of combination dose–response curves with single-agent relations has to be performed taking into account potency and shape parameters.
- Zero-interaction combination relations may be either parallel or nonparallel to single-agent relations. Whether they are parallel depends on the criteria for defining zero interaction, on the experimental design and on the mathematical expressions of the dose–response relations.
- If the zero-interaction combination dose–response relation is parallel to the single-agent relation, then a statistically significant deviation from parallelism indicates an interaction. Combination relations which are parallel but do not display the correct potency shift have to be classified as interactive as well.
- If the zero-interaction combination dose–response relation is not parallel to the single-agent relation, then any deviation from the zero-interaction

combination relation in terms of potency and/or shape indicates an interaction. This includes as a special case, a combination relation parallel to a single-agent relation.

For experimental designs which are aimed at a comparison of single-agent dose–response relations with combination relations with either a fixed amount of a second agent or a fixed dose ratio. The following procedure is recommended.

- Determination of the dose–response curve for agent A by fitting an appropriate dose–response relation to the single-agent experimental data.
- Calculation of the expected dose–response curve for the combination A+B according to a particular zero-interaction criterion and experimental design. This requires, in any case, information on the shape of the dose–response relation for agent B. The calculated curve shows a potency shift and may be either parallel or nonparallel to the single-agent curve.
- Comparison of the experimental combination effects with the calculated curve adopting an appropriate statistical test. If there is a statistically significant difference the combination has to be classified as interactive. It has to be noted, however, that the extent and even the type of a possible interaction may be dose dependent.

The analysis approach described in this paper is completely general. It is not based on any assumptions about specific parameter relationships, linear or parallel dose–response relations or experimental designs. It can be applied to any other dose–response relations, including cases, in which the relations of the agents used in a combination experiment have to be described by different mathematical expressions.

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