

## Evaluation of interaction in olfactory and taste mixtures

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**Abstract.** Models for the evaluation of interaction in olfactory and taste mixtures are compared to the isobole approach, which is widely used in other fields of biomedical research. Analogies and differences are described in detail. The isobole approach has a profound theoretical basis and can be applied to all possible types of concentration–response relationships, in particular to all values of exponents in the widely used power function, and even if analytical expression of concentration–response relationships are not known. Due to this generality it leads to a substantial simplification of the evaluation procedures as compared to other methods used in taste and olfaction research. It can be applied to any number of components of a mixture. Response surface modeling and computer graphics is recommended in appropriate cases because it can provide information on the concentration dependence of interaction. Even though it has to be pointed out that there is no general consensus on the most appropriate approach for the evaluation of interaction between biologically active agents so far, we are led to the conclusion that good arguments can be made in supporting the application of the isobole method for taste and olfactory mixtures.

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### Introduction

Interactions between drugs, carcinogens, environmental pollutants, X-rays, odorants, taste stimuli, etc., are of fundamental importance and practical interest in many branches of biomedical research. Further progress in this field requires the thorough evaluation of the type and extent of interaction. Unfortunately, there is widespread confusion about definitions, terminology and methods; for a review see Berenbaum (1989). However, the isobole approach is a powerful method in assessing interaction between biologically active agents and is thus widely used. M.C. Berenbaum has shown that it does not require any assumptions about the shapes of relations between doses or concentrations and effects or responses of single agents (concentration–response relationships) and of the underlying mechanism (Berenbaum, 1985). Although this method has been occasionally applied to olfactory and taste mixtures, other approaches are widely used in this field (Laing *et al.*, 1989). The isobole method exclusively uses concentrations, effects and empirical concentration–effect relationships and is thus completely independent of the mechanism of interaction. It is an empirical approach. This is an advantage for several reasons. It can be applied to systems for which mechanistic information is not available. Given that there is some knowledge of the mechanism the results do not depend on the usual progressive modifications of the mechanism. Furthermore, one and the same evaluation procedure can be applied to different branches of biomedical research in which completely different mechanisms may be relevant. On the other hand, the isobole approach does not explain, of course, what the reason is for any interaction. Therefore, the most appropriate way for analyzing interaction between biologically active agents is to combine empirical and mechanistic models (Ennis, 1991; Sühnel, 1992b). The aim of this work is to compare the isobole approach with other empirical models used in the field of olfaction and taste research. The terms isobole approach or isobole method as used here stand both for doing experiments in a particular manner suitable for being

evaluated by means of the isobole criterion and for the various theoretical evaluation procedures based on this criterion.

### The isobole method

Within the isobole method the reference point of no interaction or zero interaction is defined by the 'sham combination' of two amounts of one and the same agent (Loewe and Muischnek, 1924; Berenbaum, 1985, 1989). The basic relationship of this approach is the isobole equation (isobole criterion):

$$(c_A/C_A) + (c_B/C_B) = I \quad (1)$$

The quantities  $c_A, c_B$  are the concentrations or doses of agents A and B used in combination and  $C_A, C_B$  are the concentrations of A and B that would individually produce the same magnitude of effect as the combination.  $I$  stands for the index of interaction. The isobole equation can easily be extended to more than two agents. The discussion in this paper is, however, restricted to the case of two agents. Interaction indices greater than 1 indicate antagonism, smaller than 1 synergism and equal to 1 zero interaction. Readers who prefer other terms such as inhibition or additivity should simply replace the terms used in this work by their terms. This is correct as long as the mathematical criterion used is not affected. However, the term additivity is misleading as it might imply that addition of effects corresponds to the case of zero interaction. It will be shown below that this is only correct for linear concentration–response relationships. Berenbaum has shown that equation (1) holds irrespective of the shapes of dose–response relationships for single agents provided they are monotonic (Berenbaum, 1985, 1989). We have recently confirmed this claim by further arguments. In addition, we have shown that by using so-called zero interaction response surfaces the isobole approach can also be applied to non-monotonic dose–response curves (Sühnel, 1992b). 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 axes the doses of agents A and B are plotted, and which displays lines of equal biological effect, the isoboles. Given that 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. Recently, several three-dimensional evaluation procedures based on the isobole approach were proposed (Greco *et al.*, 1990; Prichard and Shipman, Jr, 1990; Sühnel, 1990, 1992a,b,c; Baumgart *et al.*, 1991). One of these useful new tools is the zero interaction response surface (Sühnel, 1992a). Zero interaction response surfaces have interaction indices  $I=1$  for all dose combinations or linear isoboles for all effect levels. They can be applied both for evaluating combination experiments and for comparing the empirical isobole approach with mechanistic models. By means of current computer graphics facilities this procedure is now easily amenable to routine application.

In the following the generation of zero interaction response surfaces is briefly described. As already noted, the isobole equation is completely independent of a

particular type of concentration–response relationships. However, if special relationships are widely used in a particular field it makes sense to combine the isobole equation with these relations. The quantities  $C_A, C_B$  represent concentrations of single agents A and B which exhibit the same effect as the combination. This means it holds  $E_{AB}^X(C_A^X, C_B^X) = E_A^X(C_A^X) = E_B^X(C_B^X)$  for a certain effect level  $X$  per definition. Recasting the concentration–response relationships after the concentrations, inserting the corresponding expressions into the denominator of the isobole equation, and replacing both the effects  $E_A$  and  $E_B$  of the single agents A and B by the effect of the combination  $E_{AB}$  thus gives a mathematical expression which interrelates  $E_{AB}$ , with  $c_A, c_B$  and  $I$ . In order to underline the completely general nature of this procedure, the character  $E$  is used for the effects instead of more special characters like  $R$  or  $\Psi$ . One can now proceed in setting the interaction index  $I$  equal to 1. In this manner an expression for a response surface is obtained for which the corresponding isobologram shows exclusively straight lines or, in other words, for which all combinations have an interaction index of 1 and display zero interaction. A response surface of this type is called, according to our proposal, zero interaction response surface  $E_{AB}^0$  (Sühnel, 1992a). On the other hand, one can replace  $E_{AB}$  by any other response surface function with the same concentration–response relationships of single agents and obtain a generalization of the interaction index  $I$ , which is according to our proposal called interaction function  $I(c_A, c_B)$  (Baumgart *et al.*, 1991; Sühnel, 1992a). If the concentrations in both the numerator and the denominator are replaced in the isobole equation, one arrives at a relationship between  $E_{AB}$ ,  $E_A$ ,  $E_B$  and  $I$ . In this work the focus is on zero interaction response surfaces.

In the following, the corresponding relationships are derived for concentration–response relationships widely used in taste and olfaction research: the power function

$$E(c) = (c/c^0)^n \quad (2)$$

where  $c^0$  is the reference concentration and  $n$  is the exponent, and the Beidler equation (Beidler, 1954)

$$E(c) = E_{\max} [cK/(1+cK)] \quad (3)$$

where  $E_{\max}$  is the maximum effect and  $K$  is the association constant of the stimulus. Note that this methodology can easily be carried over to any other concentration–response relationships. In the following the quantities defined above carry subscripts A or B, which simply means that they refer to agents A or B.

The following equations are obtained for the power function:

$$(c_A/\{c_A^0[E_{AB}(c_A, c_B)]^{1/n_A}\}) + (c_B/\{c_B^0[E_{AB}(c_A, c_B)]^{1/n_B}\}) = I \quad (4)$$

$$[E_A(c_A)/E_{AB}(c_A, c_B)]^{1/n_A} + [E_B(c_B)/E_{AB}(c_A, c_B)]^{1/n_B} = I \quad (5)$$

If  $E_{AB}$  (or  $E_{AB}^0$  for  $I=1$ ) is to be calculated, the equations have to be solved by iteration. We have written the computer program COMBITOOL which solves the

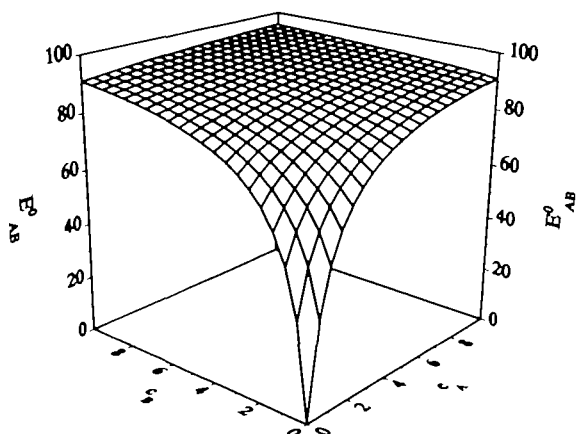


Fig. 1. Response surface for the Beidler mixture equation (zero interaction response surface,  $K_A=K_B=1$ ,  $E_{max}=100$ ; all graphs were generated using the graphics software GRAFTOOL, version 3.3, 3-D Visions Corporation).

corresponding implicit equations for several types of concentration–response relationships. For  $n_A=n_B \neq 1$  and  $I=1$  equations (4) and (5) simplify to

$$E^0_{AB}(c_A, c_B) = [(c_A/c^0_A) + (c_B/c^0_B)]^n \quad (6)$$

$$E^0_{AB}(c_A, c_B) = \{[E_A(c_A)]^{1/n} + [E_B(c_B)]^{1/n}\}^n \quad (7)$$

For  $n=1$  the expected effect of the combinations for the case of no interaction is simply the sum of the effects of single agents. Note that this is only correct for linear concentration–response relationships.

The following relationships are obtained for the Beidler equation (given  $E_{max}$  is equal for A and B):

$$\{[E_{max}/E_{AB}(c_A, c_B)] - 1\}(c_A K_A + c_B K_B) = I. \quad (8)$$

For  $I=1$  one obtains from equation (8)

$$E^0_{AB}(c_A, c_B) = E_{max}[(c_A K_A + c_B K_B)/(1 + c_A K_A + c_B K_B)]. \quad (9)$$

Equation (9) is identical to the Beidler mixture equation (Beidler, 1962), which means that this equation represents a zero interaction response surface. The relationship between the single-agent effects and the combined effect for the case of zero interaction is given by

$$E^0_{AB}(c_A, c_B) = E_{max} \{ \{ [E_A(c_A)/[E_{max} - E_A(c_A)]] + [E_B(c_B)/[E_{max} + E_B(c_B)]] \} / \{ (1 + [E_A(c_A)/[E_{max} - E_A(c_A)]] + [E_B(c_B)/[E_{max} - E_B(c_B)]] \} \}. \quad (10)$$

Computer graphics can be used to display the response surfaces. The contour plot of a particular response surface corresponds to the isobologram. In Figure 1 the response

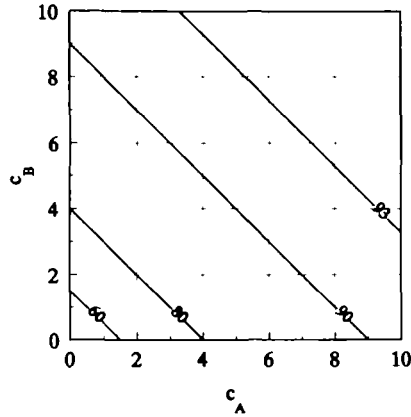


Fig. 2. Isobologram for the response surface shown in Figure 1.

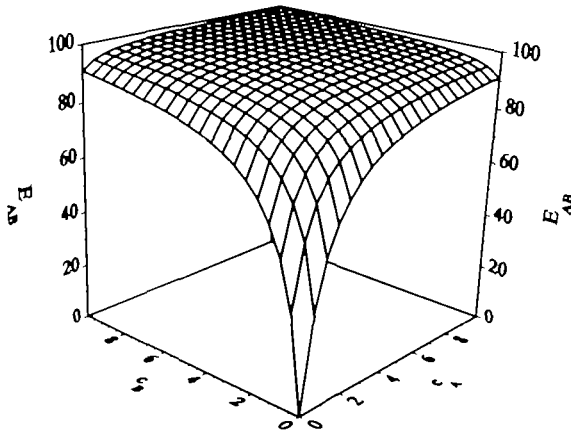


Fig. 3. Response surface for the separate-site model ( $K_A = K_B = 1$ ,  $E_{\max} = 100$ ).

surface and in Figure 2 the isobologram for the Beidler mixture equation are shown. As already noted, this surface shows zero interaction according to the isobole criterion throughout. On the other hand, the so-called separate-site model proposed by Jakinovich (1982) and McBride (1988).

$$E_{AB}(c_A, c_B) = E_A(c_A) + E_B(c_B) - \{[E_A(c_A) E_B(c_B)]/E_{\max}\} \quad (11)$$

in which for the relationship between concentration and effect of single agents the Beidler equation (3) is also used, is synergistic throughout (Figures 3 and 4), even though the single-agent concentration-response relationships for the Beidler mixture model and for the separate-site model are identical. At this point it has to be mentioned that equation (11) corresponds to the case of so-called independence. There are researchers who prefer to adopt equation (11) as criterion for the case of no interaction (Pösch, 1991). Berenbaum (1989) has shown that equation (11) and the isobole method yield only identical results

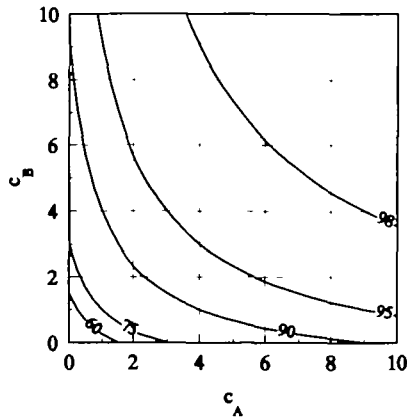


Fig. 4. Isobologram for the response surface shown in Figure 3.

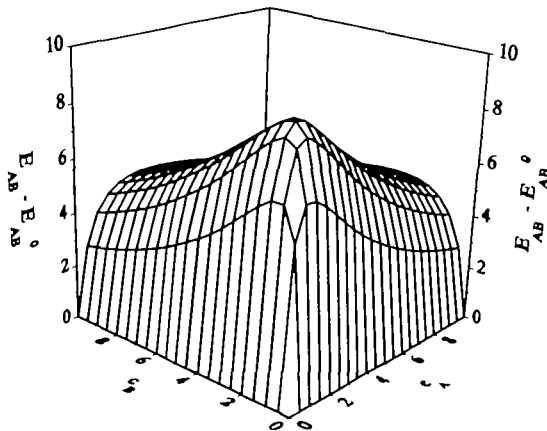


Fig. 5. Difference response surface for the separate-site model and the Beidler mixture model.

for exponential concentration – response curves. In all other cases the results are different (see also Sühnel, 1992a).

A very useful representation is a so-called difference response surface which shows the difference of two surfaces (Prichard and Shipman, Jr, 1990; Sühnel, 1992a). If one of these surfaces represents a zero interaction response surface, the difference response surface indicates directly regions of different extent or even type of interaction. The difference surface for the separate-site model and the Beidler mixture model is shown in Figure 5. One can again see that according to the isobole criterion the separate-site model is synergistic throughout with a maximum of synergism for  $c_A = c_B = 2$  (Figure 6). Note that in this model the extent of interaction is dependent on the concentrations.

**Models for the evaluation of interaction in olfactory and taste mixtures**

In this section models used for the evaluation of interaction in taste and olfactory mixtures are described in brief and compared to the isobole approach. It is beyond the scope

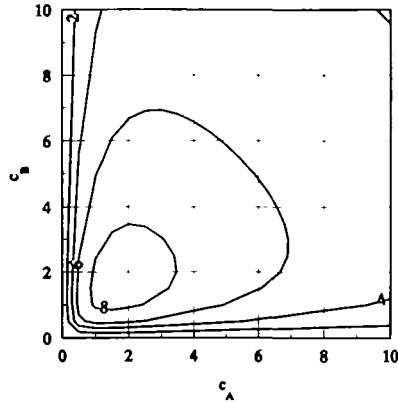


Fig. 6. Contour plot for the difference response surface shown in Figure 5.

of this work to give a comprehensive overview of all models proposed. Note, that the methodology described can easily be carried over to other models.

In 1973 Berglund *et al.* suggested that the effect of an olfactory mixture should be calculated from the effects of single agents by means of vector addition (Berglund *et al.*, 1973). Since then this vectorial model has been subject to several modifications, for a review see Laffort (1989). The most sophisticated of the vectorial models, the  $\Gamma$ -model proposed by Laffort *et al.* (1989), is described in the following. This approach is based on the power function for single agent concentration-response relations. In accordance with the nomenclature used by these authors the various quantities are not written as functions of  $c_A$  and  $c_B$ . Note, however, that they are functions of  $c_A$  and  $c_B$ . In other words, the following equations should be viewed as a shorthand notation in which  $\Gamma$  stands for  $\Gamma(c_A, c_B)$ ,  $E_A$  represents  $E_A(c_A)$  and so on. The index of interaction  $\Gamma$  is calculated as follows:

$$\Gamma = (1 + \cos\alpha_U)/(1 + \cos\alpha_{UPL2}) \quad (12)$$

$$\cos\alpha_U = (E_{AB} - E_A - E_B)/[2(E_A E_B)^{1/2}] \quad (13)$$

$$\cos\alpha_{UPL2} = (\cos\alpha_A E_A + \cos\alpha_B E_B)/(E_A + E_B) \quad (14)$$

$$\cos\alpha_A = [1 - P^{n_A} - (1 - P)^{n_A}]/[2P^{n_A/2}(1 - P)^{n_A/2}] \quad (15)$$

$$P = (c_B/c_B^0)/[(c_A/c_A^0) + (c_B/c_B^0)] \quad (16)$$

The quantity  $\cos\alpha_B$  is calculated in an analogous manner as  $\cos\alpha_A$ . According to this approach, values of  $\Gamma > 1$  indicate synergism,  $\Gamma < 1$  antagonism and  $\Gamma = 1$  zero interaction. Its main advantage over more simple procedures based on the vector model is that it gives  $\Gamma$ -values of 1 for the combination of an agent with itself.

Moreover, Patte and Laffort (1979) have proposed a so-called  $\sigma$ ,  $\tau$ -diagram. The quantities  $\sigma$  and  $\tau$  are calculated as follows:

$$\sigma = E_{AB}/(E_A + E_B) \quad , \quad (17)$$

$$\tau = E_B/(E_A + E_B) \quad . \quad (18)$$

The quantity  $\sigma$  is like  $\Gamma$  a measure of interaction.

Further interaction parameters were proposed by Hyman and Frank (1980), the independent component index *ICI*

$$ICI = E_{AB}/(E_A + E_B) \quad (19)$$

and the mixture discrimination index *MDI*

$$MDI = E_{AB}/E_{AB}^0 \quad (20)$$

*ICI* and *MDI* were suggested assuming power function dose-response relationships and  $n_A = n_B$ . In this case,  $E_{AB}^0$  is given by equation (6). The *ICI* index is, of course, identical to  $\sigma$ .

How are the indices  $\Gamma$ ,  $\sigma$ , *ICI* and *MCI* related to the index of interaction *I* of the isobole equation? For all of these quantities it was explicitly or tacitly assumed that the single-agent concentration-response relationships are the power function. To simplify the analysis let us first assume that  $n_A = n_B = n$  holds. In this case  $\Gamma$  is given by

$$\Gamma = [2(E_A E_B)^{1/2} + E_{AB} - E_A - E_B] / [2(E_A E_B)^{1/2} + E_{AB}^0 - E_A - E_B]. \quad (21)$$

Note that  $E_{AB}^0$  can be calculated according to equation (6). With

$$K = 2(E_A E_B)^{1/2} - E_A - E_B \quad (22)$$

$\Gamma$  can be written as

$$\Gamma = (K + E_{AB}) / (K + E_{AB}^0) \quad (23)$$

Inserting *MDI* into equation (23) leads to

$$\Gamma = [K + MDI E_{AB}^0] / [K + E_{AB}^0] \quad . \quad (24)$$

It can be seen that  $\Gamma$  and *MDI* are identical provided *K* equals 0. This holds exactly for  $E_A = E_B$ . For  $E_A \neq E_B$  there is a slight difference. To give an example for  $E_A = 22.7$  and  $E_B = 12.3$  the quantity *K* amounts to 1.58, which yields *MDI*=0.67 and  $\Gamma$ =0.69. Note that  $\Gamma = MDI = 1$  holds for any values of *K*. For  $n_A = n_B = n$  there is a simple relation between  $\Gamma$ , *MDI* and *I*. According to equation (5) it holds

$$E_{AB} = [(E_A)^{1/n} + (E_B)^{1/n}]^n / I^n = E_{AB}^0 / I^n \quad (25)$$

This leads to

$$MDI = [K(\Gamma - 1)/E_{AB}^0] + \Gamma = 1 / I^n \quad . \quad (26)$$

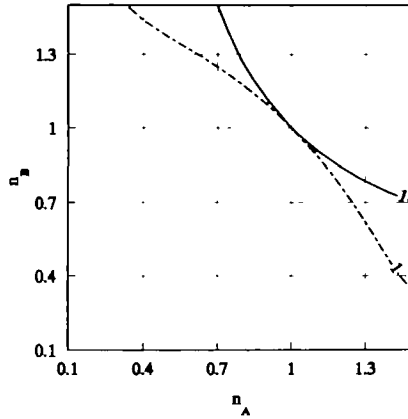


Fig. 7. Contour plots for the dependence of  $\Gamma$  (dashed line) and  $I$  (solid line) on the power law exponents  $n_A$  and  $n_B$  for  $E_A = E_B = 5$  and  $E_{AB} = 10$ .

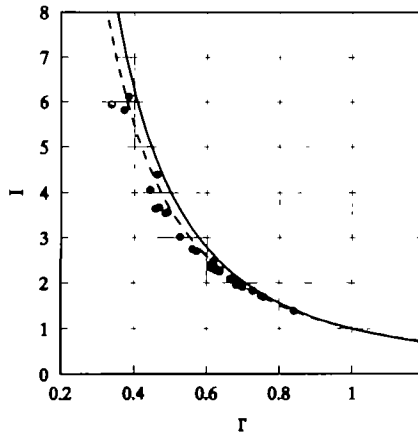


Fig. 8. Relationship between  $I$  and  $\Gamma$  for psychophysical data of olfactory mixtures with geraniol and cineole reported by De Wijk (1989, and personal communication, 1991). [Subject 1;  $n_{\text{geraniol}} = 0.50$ ,  $n_{\text{cineole}} = 0.54$ ; the solid line ( $n = 0.54$ ) and the dashed line ( $n = 0.50$ ) were calculated according to equation (26) for  $K=0$ .]

As already mentioned, values of  $I$  smaller than 1 and values of  $MDI$  or  $\Gamma$  larger than 1 indicate synergism. It is, however, important to note that for  $n_A = n_B = n$  the indices  $MDI$ ,  $\Gamma$  and  $I$  lead to identical results in the case of zero interaction. Is this also true for  $n_A \neq n_B$ ? Unfortunately, this cannot be shown by means of simple analytical relations. In Figure 7 a contour plot is shown, which displays the dependence of  $I$  and  $\Gamma$  on  $n_A$  and  $n_B$  for a particular combination of  $E_A$ ,  $E_B$  and  $E_{AB}$ . Note that other combinations of the effects yield similar results. As already noted, for  $n_A = n_B$   $\Gamma$  and  $I$  give identical results for the case of zero interaction. However, for  $n_A \neq n_B$  this is no longer true. For  $n_A, n_B$ -combinations in the area between the solid and the dashed lines an interaction classified as synergistic by the  $\Gamma$ -model is antagonistic according

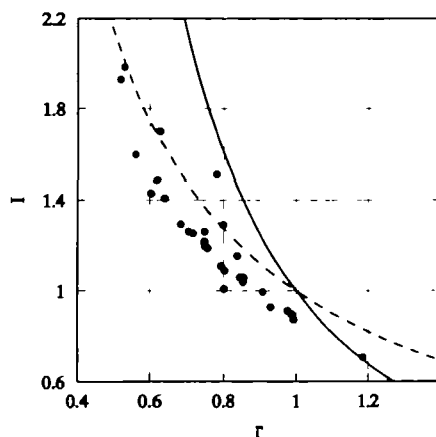


Fig. 9. Relationship between  $I$  and  $\Gamma$  for psychophysical data of olfactory mixtures with geraniol and cineol reported by De Wijk (1989, and personal communication, 1991). [Subject 3;  $n_{\text{geraniol}} = 0.92$ ,  $n_{\text{cineol}} = 0.47$ ; the solid line ( $n = 0.47$ ) and the dashed line ( $n = 0.92$ ) were calculated according to equation (26) for  $K=0$ .]

to the isobole approach and vice versa. In other words, the  $\Gamma$ - and the isobole models lead for  $n_A \neq n_B$  to different results and the question arises how marked this difference is.

In Figures 8 and 9 the relationships between  $I$  and  $\Gamma$  are shown for experimental psychophysical data on olfactory mixtures of geraniol and 1,8-cineol with almost equal and different power function exponents (De Wijk, 1989, and personal communication, 1991). The solid and dashed lines represent the theoretical relationships for  $K=0$  and a particular power function exponent (equation 26). Note that this equation was derived under the assumption that the power function exponents of the mixture components are equal. It can be seen from Figure 8 (almost equal power function exponents) that the effect of  $K$  is rather small. On the other hand, Figure 9 clearly shows, that for different power function exponents there is a marked difference to the relationship between  $I$  and  $\Gamma$  derived for equal power function exponents, equation (26). This is exactly the result expected from the data shown in Figure 7. In spite of these differences it is certainly correct to say that in the majority of cases the qualitative classification of an interaction as synergistic, antagonistic or zero-interactive will be the same with the  $\Gamma$ -model and the isobole approach. In a more quantitative sense, however, similar results can only be expected for equal or almost equal power function exponents.

From the point of view of the isobole approach, the quantities  $\sigma$  and  $ICI$  only give correct results for  $n_A = n_B = 1$ . Laffort (1989) has pointed out that a deviation from the straight horizontal line in the  $\sigma$ ,  $\tau$ -diagram may be either due to interaction or to power function exponents  $n \neq 1$ . Again, adopting the isobole approach leads to the conclusion that  $\sigma$  values which deviate from 1 due to power function exponents  $n \neq 1$  do not indicate interaction. One would, of course, prefer to have an approach at hand which does not mix up the extent of interaction and variations in the power function exponents. To this end it is useful to define this index as

$$\Sigma = E_{AB} / E_{AB}^0 \quad (27)$$

The quantity  $E_{AB}^0$  stands for the expected effect in the case of zero interaction. Note that  $E_{AB}^0$  can be calculated for all possible dose-response relationships. Using power functions for  $n_A = n_B = 1$   $E_{AB}^0$  is simply the sum of  $E_A$  and  $E_B$ , see equation (7). In this case  $\Sigma$  corresponds to  $\sigma$  or  $ICI$ . For  $n_A = n_B = n$ , equation (5),  $\Sigma$  corresponds to the  $MDI$  index. For  $n_A \neq n_B$   $E_{AB}^0$  has to be calculated according to equation (5) ( $I=1$ ) by numerical iteration. Using  $\Sigma$  instead of  $\sigma$  leads, for the case of no interaction, to a horizontal straight line at  $\Sigma=1$  in the  $\Sigma, \tau$ -diagram irrespective of the values of the power function exponents.

Furthermore, our analysis shows that the indices  $ICI$  and  $MDI$  proposed by Hyman and Frank (1980) are not different criteria for assessing zero interaction, which would be rather confusing, but represent particular cases of a more general criterion.  $ICI$  can only be applied to linear single-agent dose-response relations and  $MDI$  only to power function dose-response relationships with  $n_A = n_B = n$ .

As already noted there are further approaches for evaluating interaction in olfactory and taste mixtures. It is not possible to discuss all these approaches in greater detail here. On the other hand, a short discussion of the following procedures is appropriate: the substitution model of Moskowitz (1973), the summated comparison rule, the equimolar comparison rule (De Graaf and Frijters, 1987), and the factorial plot comparison (McBride, 1986). In the latter three cases the terminology of De Graaf and Frijters (1988) was used.

The substitution model of Moskowitz (1973) claims that the expected combination effect for the case of zero interaction is given by equation (28)

$$E_{AB}^0(c_A, c_B) = k_A \{c_A + [k_B c_B^{n_B} / k_A]^{1/n_A}\}^{n_A} \quad (28)$$

This equation simplifies to

$$E_{AB}^0(c_A, c_B) = k_A [c_A + (k_B/k_A) c_B]^n \quad (29)$$

for  $n_A = n_B = n \neq 1$  and to

$$E_{AB}^0(c_A, c_B) = k_A c_A + k_B c_B \quad (30)$$

for  $n = 1$ . For comparing these expressions to the isobole approach first note that the power function used by Moskowitz (equation 31),

$$E(c) = k c^n \quad (31)$$

is slightly different to equation (2). Therefore, the equations describing the zero interaction response surface are slightly different to equations (4) and (6). They read

$$\{c_A/[E_{AB}^0(c_A, c_B)/k_A]^{1/n_A}\} + \{c_B/[E_{AB}^0(c_A, c_B)/k_B]^{1/n_B}\} = 1 \quad (32)$$

and

$$E_{AB}^0(c_A, c_B) = [(k_A^{1/n} c_A) + (k_B^{1/n} c_B)]^n \quad (33)$$

One can easily see that the expressions of the substitution model and of the isobole approach are only identical for  $n=1$ . However, for  $n_A=n_B=n$  the equations lead also to identical results given  $k_A=k_B$  holds. This means that both the approach used by Moskowitz and, of course, the isobole approach lead to correct results for the mixture of two amounts of one and the same agent. Note that the thoughtful analysis of the substitution model by Frijters and Oude Ophuis (1983) led to the very same results.

The summated comparison rule gives a predicted effect of

$$E_{AB}(c_A, c_B) = (c_A/c_A^0)^{n_A} = (c_B/c_B^0)^{n_B} \quad (34)$$

In this case the result is only identical to the isobole criterion for  $n_A=n_B=1$  that means for linear concentration – response relationships.

As far as the equimolar comparison rule (De Graaf and Frijters, 1987) is concerned one has first to define zero interaction. Let us assume that a mixture displays zero interaction if the combination effect is somewhere between the single-agent effects. According to equation (6) the isobole criterion leads for power functions with  $n_A = n_B = n$  to

$$E_{AB}(c_A/2, c_B/2) = [(1/2)(c_A/c_A^0 + c_B/c_B^0)]^n \quad (35)$$

One can easily see that according to this equation the combination effect for half of the single-agent concentrations  $E_{AB}(c_A/2, c_B/2)$  is always between  $E_A(c_A)$  and  $E_B(c_B)$ . One can further see that this statement is independent of the value of  $n$ . For  $n_A \neq n_B$  there is no explicit expression for  $E_{AB}$ , see equation (4). Therefore, it is not easy to give a definite proof of the assumption that  $E_{AB}(c_A/2, c_B/2)$  calculated by means of the isobole criterion should have a value within the range covered by  $E_A(c_A)$  and  $E_B(c_B)$ . However, we have performed a great deal of calculations for different parameter sets using our program COMBITOOL, which solves implicit equations. In all cases it was found that indeed  $E_{AB}(c_A/2, c_B/2)$  had a value within the range covered by  $E_A(c_A)$  and  $E_B(c_B)$ . Hence, it can be concluded that for power function dose – response relationships the equimolar comparison rule and the isobole criterion lead to analogous results also for the case of different power function exponents. Note, however, that according to the equimolar comparison rule the effect range classified as zero-interactive is rather large given the difference between  $E_A(c_A)$  and  $E_B(c_B)$  is large. In this case the equimolar comparison rule is not very useful and the isobole criterion should be preferred.

The factorial plot comparison (McBride, 1986) claims that zero interaction is defined by

$$E_{AB}(2c_A, 2c_B) - E_{AB}(2c_A, c_B) = E_{AB}(c_A, 2c_B) - E_{AB}(c_A, c_B) . \quad (36)$$

When inserting the corresponding expressions (see equation 6), it turns out that the factorial plot comparison and the isobole criterion yield identical results for  $n_A=n_B=1$ . In all other cases the results are different.

The essential result of this discussion is that the equimolar comparison rule is the only approach which gives results analogous to the isobole criterion, even though one

should keep in mind that this approach does not work very well for substantial differences in  $E_A$  and  $E_B$ . All the other approaches, including the  $\Gamma$ -model, give results identical to the isobole approach only for particular cases. Hence, if one adopts the view that the isobole approach provides a generally valid criterion for defining zero interaction then this approach is the method of choice for assessing the interaction of biologically active agents. It represents a general procedure which contains a few of the methods used so far as particular cases. It defines the range of applicability of these methods precisely and thus leads to a generalization and simplification of evaluation procedures. The isobole approach can be applied in different versions which all lead, if appropriately applied, to the same results (Sühnel, 1992a). The index of interaction  $I$  can easily be calculated for any concentration combination provided the single-agents concentration–response relationships are known (see equations 1, 4, 5, 8). An additional possibility is to generate graphs of the zero interaction response surface (see Figures 1 and 10, and equations 4, 6, 9, 32). A deviation of an experimental data point from this surface directly indicates synergism or antagonism. A difference response surface represents the difference between an actual response surface and the corresponding zero interaction response surface. The zero line indicates zero interaction in the corresponding contour plot (see Figures 5 and 6). Finally, the interaction function, which represents a generalization to the interaction index, can be calculated. This approach is not used in this paper. It requires the calculation of indices of interaction for all dose combinations. Then a ‘surface’ can be fitted to these data, which represents the interaction function. A contour plot of the interaction function (iso-interaction diagram) displays lines of equal interaction. An application of this procedure is described by Baumgart *et al.* (1991). Whereas the first two methods can be applied to cases in which only a very small number of dose combinations were studied, the other two approaches require an experimental design appropriate for a surface modeling of response data or interaction indices. Upon fitting response surfaces to experimental data one may run into difficulties. Given the number of combination experiments is small, this is rather the exception than the rule, the results obtained may be heavily dependent on the mathematical expression used for the fitting procedure. On the other hand, the generation of zero interaction response surface is rather simple. It requires a non-linear regression for the single-agent concentration–response relationships. This is a simple task as compared to non-linear regression of response surfaces. Then the zero interaction response surfaces is calculated using the parameters of the single-agent concentration–response relationships and the appropriate expression for  $E_{AB}^0$ . For power functions this is equation (4) with  $I=1$ . The experimental data can directly be compared with this surface. Any deviation indicates interaction.

### **Is the interaction in olfactory or taste mixtures concentration dependent?**

It is one of the basic tenets of the theory of interaction between biologically active agents that extent and even type of interaction may be dependent on dose or concentration (Berenbaum, 1989). This seems to be well-known in the field of pharmacology. However, the separate-site model (Figures 3 and 4), is also one example of this fact. Therefore, it was rather surprising to learn that in taste and olfaction research procedures are used which assume that interaction is independent of concentration. For example,

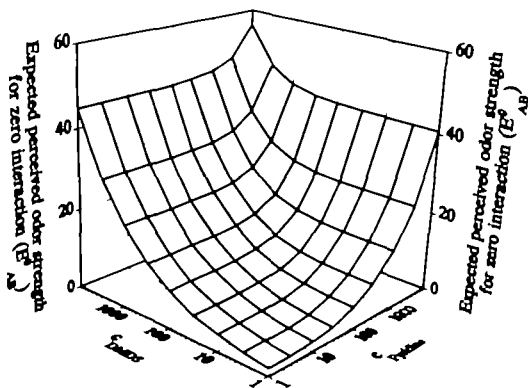


Fig. 10. Zero interaction response surface  $E_{AB}^0$  (expected perceived odor strength for the case of zero interaction according to the isobole approach) for psychophysical data for the mixture of pyridine and dimethyldisulfide (DMDS) reported by Olsson (1986). [ $E_{AB}^0$  was calculated by means of equation (4) with  $I=1$  and using the following power functions; pyridine:  $\log E = -0.15 + 0.44 \log c$ ,  $E = (c/2.19)^{0.44}$ ; DMDS:  $\log E = 0.29 + 0.34 \log c$ ,  $E = (c/0.14)^{0.34}$ ;  $c$  in ppb.]

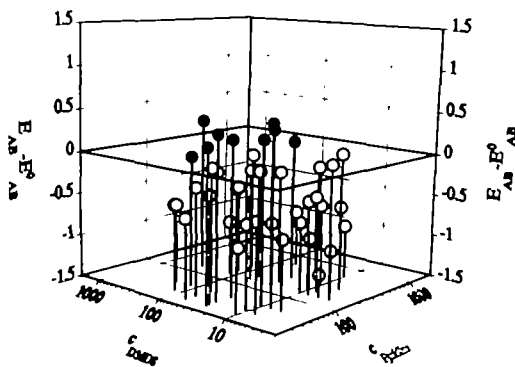


Fig. 11. Difference  $E_{AB} - E_{AB}^0$  for olfactory mixtures of pyridine and DMDS (Olsson, 1996). [ $E_{AB}$  stands for the experimental data and  $E_{AB}^0$  for the expected effect for zero interaction calculated as described in the legend to Figure 10; full circles indicate positive values and 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;  $c$  in ppb.]

the vector model in its original version contains only one interaction parameter for a pair of substances independent of concentration. A more subtle approach which is, however, also based on the assumption of concentration independent interaction is to determine interaction parameters ( $\cos \alpha$ ,  $\Gamma$ ) for all dose combinations and then to calculate mean values of these parameters. They are then, in turn, used to predict effects of unknown combinations (Laffort, 1989). Note that the same could also be done with the index of interaction  $I$  of the isobole method. According to our view, it is obvious that this approach is only correct if it was checked before that in the concentration range under study there is, indeed, no variation in type or extent of interaction, unless there are any arguments which prove that there is no variation in interaction with concentration

for olfactory and taste mixtures, in general. In the following, this question is posed to two experimental data sets: psychophysical data for the mixture of pyridine and dimethyldisulfide (DMDS) reported by Olsson (1986), and the data reported by De Wijk (1989, and personal communication, 1991) already mentioned.

The single-agent concentration-response relationships given by Olsson were used to construct the corresponding zero interaction response surface (pyridine:  $\log E = -0.15 + 0.44 \log c$ ,  $E = (c/2.19)^{0.44}$ ; DMDS:  $\log E = 0.29 + 0.34 \log c$ ,  $E = (c/0.14)^{0.34}$ ;  $c$  in ppb; Figure 10). The experimental data points could have been inserted into this surface. A better representation, however, is shown in Figure 11. Here, the difference between the actual response  $E_{AB}$  and the expected response for zero interaction  $E^0_{AB}$  is shown. Obviously, the deviation of the measured odor strength from the zero interaction response surface is rather small. The maximum deviations are +0.412 and -1.312. There is no indication of a concentration-dependent interaction. Therefore, calculating the mean values of interaction parameters and predicting the effects of other concentration combinations using these mean values is justified. The following mean values are obtained:  $I = 1.13 \pm 0.17$  (equation 5),  $E_{AB} - E^0_{AB} = -0.33 \pm 0.43$ ,  $\Sigma = E_{AB}/E^0_{AB} = 0.96 \pm 0.06$ ,  $\Gamma = 0.94 \pm 0.05$ . All these values indicate slight antagonism. However, the standard deviations include the zero interaction case. Due to the almost equal power function exponents and due to the fact that the interaction parameters are in the vicinity of 1, the isobole approach and the  $\Gamma$  model give the same results. Therefore, the interpretation given by Laffort (1989) is confirmed in almost all points by this analysis. Note, however, that the  $\Sigma$  values are in the vicinity of 1, whereas the  $\sigma$  values given by Laffort (1989) for the same data set are much smaller. This is due to the fact that  $E^0_{AB}$  is calculated by Laffort by the sum of  $E_A$  and  $E_B$ , whereas the  $E^0_{AB}$  value used for  $\Sigma$  is calculated according to equation (4) for  $I=1$ .

For the data of De Wijk (1989, and personal communication, 1991) the determination of the power function parameters was repeated by means of the nonlinear regression of the STATGRAPHICS software package (version 3.0). De Wijk obviously used the logarithmic relationships for parameter estimation. It is now common practice to work with untransformed data. Nevertheless, there are only minor differences in the parameters obtained

{subject 1: geraniol- $E = [c/(0.51 \pm 0.07)]^{(0.50 \pm 0.009)}$ , cineol- $E = [c/(0.80 \pm 0.04)]^{(0.54 \pm 0.004)}$ ;  
subject 3: geraniol- $E = [c/(29.43 \pm 3.82)]^{(0.92 \pm 0.03)}$ , cineol- $E = [c/(0.28 \pm 0.01)]^{(0.47 \pm 0.003)}$ ;  
subject 4: geraniol- $E = [c/(20.43 \pm 4.39)]^{(0.91 \pm 0.04)}$ , cineol- $E = [c/(32.00 \pm 0.54)]^{(1.10 \pm 0.006)}$ ;  
 $c$  in ppb}.

In comparison with the Olsson data the mean values of  $\Gamma$  and  $I$  show a much larger standard deviation: subject 1:  $I=2.87 \pm 1.29$ ,  $\Gamma=0.59 \pm 0.13$ ; subject 3:  $I=1.23 \pm 0.30$ ,  $\Gamma=0.78 \pm 0.15$ ; subject 4:  $I=1.51 \pm 0.29$ ,  $\Gamma=0.68 \pm 0.15$ . Interestingly, the deviation from the mean value is different for different concentrations. This can be most clearly seen for subject 4. The concentrations used by De Wijk cover only a very small area of the two-dimensional concentration range. Therefore, a two-dimensional representation is used in this case. In Figures 12 and 13 the  $\Gamma$  and  $I$  values are plotted versus the sum of the geraniol and cineol concentrations. The solid line indicates the mean values. It is obvious that in the low dose range the  $\Gamma$  values are

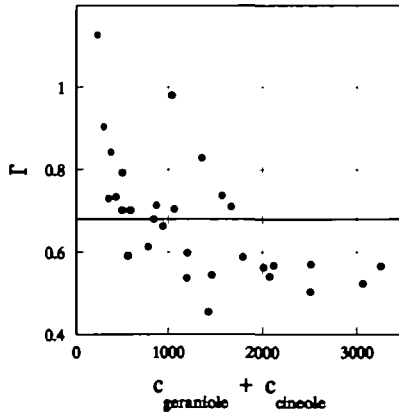


Fig. 12. Dependence of the  $\Gamma$  index on the sum of concentrations of geraniol and cineole for psychophysical data reported by De Wijk (1989, and personal communication, 1991) (subject 4).

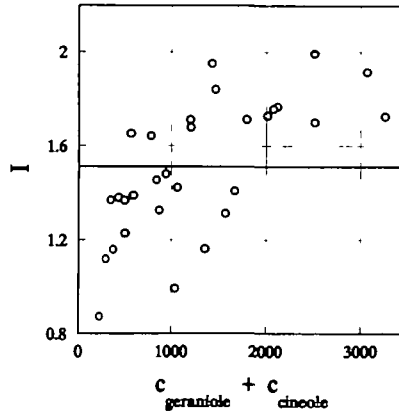


Fig. 13. Dependence of the index of interaction  $I$  on the sum of concentrations of geraniol and cineole for psychophysical data reported by de Wijk (1989, and personal communication, 1991).

consistently larger than for higher doses. On the other hand, the  $I$  values are generally smaller in the low dose range and larger for higher doses. According to our view these results indicate that the extent of antagonistic interaction is more pronounced for the high-dose range than for the low-dose range. A similar situation refers to subject 3 even though in this case the concentration dependence of  $I$  and  $\Gamma$  is not as pronounced. On the other hand, for subject 1, antagonism is reduced in passing to higher concentrations. In any case, there is a dependence of the interaction parameters on concentration (and thus of effect) of the mixture components. As can be seen from Figures 12 and 13, using the mean values of  $I$  or  $\Gamma$  for predicting effects for unknown combinations of concentrations would lead to too low values in the low-dose range and to too high values in the high-dose range. From this analysis it has to be concluded that there may be a concentration dependent extent of interaction in olfactory mixtures.

This is also in line with the conclusions of De Wijk (1989). Therefore, the prediction of unknown combination effects using mean values of interaction indices is only appropriate if it was checked before that there is no marked concentration dependence of interaction. If there is such a dependence, the complete concentration range could possibly be subdivided into subranges with almost equal extent of interaction.

Laffort *et al.* (1989) and Laffort (1989) have criticised the application of the isobolograms, which use concentrations, because they may display irregular patterns, where, for example, the isoboles for a particular effect level cross the linear zero interaction isobole several times (see for example the data reported by Köster, 1969). However, there are other isobolograms in the field of taste and olfaction research which do not display these irregularities (Ennis, 1989; Jones and Woskow, 1964). Laffort *et al.* (1989) have calculated iso-intensity curves according to the  $\Gamma$ -model and have shown that for  $\Gamma = 1$  (zero interaction) non-linear isoboles are obtained for the case  $n_A \neq n_B$  which display in a few cases patterns which bear resemblance to the Köster isobolograms. Due to the fact that isoboles may be non-linear even though they correspond to  $\Gamma = 1$ , Laffort has introduced the terms apparent and true synergism or antagonism (inhibition). This phenomenon seems to be generally known and is usually called 'Fechner paradox' (Gregson, 1986). If one adopts the point of view of the isobole approach, the  $\Gamma$ -model does not take into account different power function exponents in an appropriate manner. Hence, in the light of the isobole approach, the iso-intensity curves calculated by Laffort *et al.* (1989) are artifacts in the case of different power function exponents. If one adopts this point of view, the occurrence of the Fechner paradox is not a consequence of the fact that perceived intensities are expressed as functions of concentrations of components, but rather of an incorrect treatment of the power function exponents in the procedures for evaluating interaction.

Furthermore, Berenbaum (1989) has pointed out that there are experimental data for which the isobole for one effect-level may indicate synergism in one concentration range and antagonism in another. Therefore, isoboles of this type cannot be excluded in general. On the other hand, 'irregular' isoboles may also be the consequence of marked data scattering. In this case, it makes no sense to construct isobolograms because they will display a great deal of artifacts. Other methods such as, for example, the comparison of experimental data with the corresponding zero interaction response surface are then more appropriate.

## Conclusions

Models for the evaluation of interaction in olfactory and taste mixtures are compared to the isobole approach, which is widely used in other fields of biomedical research. This approach has a profound theoretical basis and can be applied to all possible types of concentration – response relationships, in particular to all values of exponents in the widely used power function, and even if analytical expression of concentration relationships are not known. Due to this generality it leads to a substantial simplification of the evaluation procedures. The isobole approach provides a theoretical scheme in which alternatively concentrations and effects can be used. It can be applied to any number of components of a mixture. Response surface modeling is recommended in appropriate cases because it can provide information on a possible different extent or type of interaction in different dose ranges.

Anybody involved in the evaluation of interaction between biologically active agents should be aware of the fact that so far there is no general consensus on the most appropriate approach. However, it is felt that there are a great deal of good arguments supporting the isobole method also for the field of taste and olfaction research.

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