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ARTICLE

A general approach to the isobolographic method

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Abstract

Strict definitions and formal mathematical constructions are given to represent the main concepts of the isobolographic method as mathematical objects. In particular, a strict definition of zero interaction notion is introduced. The peculiarity of this definition is that this notion appears to depend on the dose-response function of a particular acting agent, whereas it is commonly believed that it is completely determined only by the whole set of acting agents. It is shown that without additional assumptions about the type of dose-response functions, a type of joint action of agents can be different and even opposite depending on the dose-response function of which the notion of zero interaction is considered. The only case when the concept of zero interaction is unambiguously defined and does not depend on the chosen dose-response function is the case of scale equivalence of dose-response functions of all acting agents. A theorem on the representation of the zero-interaction manifold in the case of arbitrary single-factor dose-response functions is proved. Examples of analyzing the joint action of factors using isoboles for a two-factor linear model with a cross term and a quadratic model are considered.

Keywords: Isobolographic analysis of joint action of biologically active agents; Dose-response function; Zero-interaction; Scale-invariant functions; Response surface method; Second order response model.

1. Introduction

Problems of identifying the type of joint action exhibited by several agents together emerge naturally in such sciences as toxicology, pharmacology, evidence based medicine, risk assessment, biology, and others. The phenomenon of joint action appears to be nontrivial considering the fact that the magnitude of the effect caused by a few factors does not always match the expected value of this effect computed in some way on the basis of single-factor effects. For instance, it was observed in pharmacology that a joint impact of drugs may produce a considerably different effect than could be expected based on the effect of each of the agents acting alone (Zeliger, 2011). In the treatment of the majority of serious or complicated diseases (infections, high arterial blood pressure, cardio-vascular diseases, etc.), an adequate therapy would mostly involve a combination of several medications. In

inpatient settings, combinations of several drugs are also the most widespread form of therapy. For such a combined therapy to be efficacious, it is necessary to have accurate information on how specific drugs may interact in the human organism. This information is always available in medical product leaflets in the section "Interaction with other medicinal products".

In principle, the following outcomes are possible where biologically active agents are co-administered: harmful effect caused by one or several agents, its enhancement; absence of any visible effect; and enhanced efficacy of one or several agents. Agents may interact pharmacodynamically and/or pharmacokinetically (see the specialized "Journal of Pharmacokinetics and Pharmacodynamics", and (Brody, 2018; Dumbreck *et al.,* 2015)); however, knowledge of such fine mechanisms of action is often lacking or insufficient. It is therefore reasonable to develop joint action assessment methods that would employ only observable characteristics of action expressed as values of dose and observed effects. One of the techniques of such assessment is the isobolographic method.

The isobole is a line (or a surface if there are more than two agents involved), on which the resulting function (response) has a constant value, i. e. the isobole is a response level surface. The key issue when describing the type of joint action using the isobolographic method is to determine the position of the dose combination and the magnitude of the corresponding response relative to the additivity surface corresponding to the same response level. The additivity surface is defined as a (hypothetical) surface such that for every point on it the agents in corresponding doses do not interact with respect to a given response. The additivity surface is nearly always assumed to be rectilinear, i. e. a straight line, a plane or a hyperplane in a multidimensional space. In this case, obviously, the additivity surface is defined by the points of its crossing with the axes of coordinates, i.e. the isolated doses of agents, the value of the response to which is equal to the value of the response on this surface.

Although this method was proposed around 150 years ago (the first publication is considered to be (Fraser, 1870–1871)) and it has been widely used in both practical and theoretical studies, its principal postulates still remain insufficiently rigorously formulated. The isobolographic method was first presented as a computational procedure for joint action type assessment in (Fraser, 1870–1871; Fraser, 1872; Loewe & Muischnek, 1926; Loewe *et al.,* 1927; Loewe, 1927; Loewe, 1928; Loewe, 1953; Loewe, 1957).

The further development of the method was mainly directed at extending its range of applications as summarized in the review (Berenbaum, 1989), containing an extensive bibliography on the application of the isobolographic method to specific studies. In a few studies, researchers investigated the joint action of several medications on humans with the help of isoboles (Tverskoy *et al.,* 1988; Tverskoy *et al.,* 1989b; Tverskoy *et al.,* 1989a). Further on, such studies grew in number and diversity, demonstrating the versatility of the isobolographic method in a broad variety of contexts (Atwal *et al.,* 2019; Basting *et al.,* 2019; Short & Hannam, 2019; van den Berg *et al.,* 2017).

In addition to purely practical applications, a more abstract approach was developed to answer the question under what conditions this method is generally applicable. It is also crucially important to clarify the principal notions of the method, which, as a rule, are treated as self-evident. In this regard, key works are (Loewe, 1953; Loewe, 1957) and (Berenbaum, 1977; Berenbaum, 1978; Berenbaum, 1988).

Subsequent progress in theoretical analysis and practical application of the isobolographic method has led to the emergence of numerous variants, some of which have been described in reviews (Foucquier & Guedj, 2015; García & Lage, 2013; Greco *et al.,* 1992; Greco *et al.,* 1995; Tallarida, 2000; Tang *et al.,* 2015).

Obviously, the isobolographic method may be used where the agents and the responses are continuous variables. For instance, in addition to pharmacology and toxicology, a natural area of its application could be environmental ecology, where it is essential to have a correct mathematical technique for evaluating the type of combined action of harmful agents. In large cities, where

residents are constantly exposed to a multifactorial mixture of technogenic and household pollutants, correct assessment of their joint action is a critical component of making competent management decisions.

Although this method and its variants are widely used, it is not sufficiently rigorous in some respects, which may result in an incorrect conclusion on the type of joint action considered. In particular, it is a common belief that the isobole representing absence of interaction (the so called *additivity isobole*, or *zero interaction isobole*), is geometrically a straight line (a hyperplane for many acting agents as a general case). It is shown below that it is not always the case (see Sec. 4, 5.5). At the same time, since the classification of joint action types is built relative to the zero interaction isobole, it is critically important to know the correct location of the latter. Thus, rigorous formalization of the isobolographic method is currently an important problem in the theory of combined (joint) action of factors.

The theoretical foundations of the isobolographic method were considered in (Berenbaum, 1985). In particular, the concept of zero interaction was introduced there as a replacement for the vague notion of no-interaction. In a non-strict interpretation, zero interaction is a joint action of biologically active agents which can be represented as a joint action of the same agent taken at different doses. Another important contribution of this work is the justification that the zero interaction isobole is represented by a linear manifold (a straight line, a plane, or a hyperplane). However, this justification was based on the assumption that all dose-response functions of the agents are in some sense interchangeable (see below the equality (6)).

The question of the linearity of the zero interaction isobole has a long history, and it is generally assumed that it is represented by a hyperplane. The argumentation of this fact (Berenbaum, 1985) supports this opinion. In particular, author of (Berenbaum, 1985) thought that a zero interaction isobole is always linear and argued against Loewe that the additivity isobole (in Loewe's terminology, or zero interaction isobole according to (Berenbaum, 1985)) *may differ from the rectilinear surface* for arbitrary dose-response functions. The paper (Berenbaum, 1985) supports the view that *irrespective* of the shape of the dose-response curve, zero interaction is represented by a hyperplane. It will be shown below that in the general case the zero interaction manifold is described by an equation that is not linear for arbitrary dose-response functions.

2. Some Notions and Notations

Let there be n agents which have isolated (i. e. single-factor) dose-response functions $f_i(x_i)$, $i = 1, 2, \ldots, n$. Here, x_i is a certain measure of the quantity of the acting substance. As a rule, this is a concentration in certain units, and the value of all response functions $f_i(x_i)$ has the same meaning, i. e. it is measured in the same units. For instance, it could be the amount of hemoglobin in the blood (g/L), the mass of the liver of an experimental animal (g, or g per 100 g of body weight), de Ritis coefficient (dimensionless quantity), etc. It should be emphasized that the method of presenting the dose-response relationship is not fundamental. In particular, it can be either a natural expression of the effect dependence on the dose (concentration), or an expression of the effect dependence on the fraction of the actual dose in relation to some value (for example, ED_{50}). It is important that all dose-response functions are expressed in relation to the same effect. The joint (combined) effect of these agents on a biological system is described by a multifactorial response function $Y(x_1, \ldots, x_n)$, measured in the same units as the single-factor dose-response functions $f_i(x_i)$. The single factor dose-response functions $f_i(x_i)$ and the multi-factor response function $Y(x_1, \ldots, x_n)$ are related by the equalities

$$f_i(x_i) = Y(0, \ldots, 0, x_i, 0, \ldots, 0), \quad i = 1, 2, \ldots, n \tag{1}$$

Note that the notion of multifactorial dose-response function has an important, though not

always recognized additional properties. For example, each of its arguments is characterized not only by quantity ("dose"), but also by a certain intrinsic characteristic reflecting its chemical, physical or other specific properties. Thus, it does not matter where the agent is in the list of arguments of the function Y; rather, what matters is *what kind of substance* it is. In this sense, the response function $Y(x_1, x_2, \ldots, x_n)$ is a function of not only the arguments $(x_1, \ldots, x_n)$, but also of certain "marker" of each of the arguments (for instance, chemical formula of the substance) that determine a given substance/argument.

Consequently, the dose-response function is invariant with respect to argument ordering. If we denote these markers with symbols θ_i, $i = 1, 2, \ldots, n$, then the response function will satisfy the equality

$$Y\left(x_1(\theta_1), x_2(\theta_2), \ldots, x_n(\theta_n)\right) = Y\left(P\left(x_1(\theta_1), x_2(\theta_2), \ldots, x_n(\theta_n)\right)\right), \tag{2}$$

where P is arbitrary permutation of an n-element set.

Moreover, if arguments of the multifactorial response function *relate to the same marker*, then this response function reduces to a single factor dose-response function, i. e. the following equality is satisfied

$$Y\left(x_k^{(1)}(\theta_k), x_k^{(2)}(\theta_k), \ldots, x_k^{(n)}(\theta_k)\right) = Y\left(0, \ldots, 0, \sum_{j=1}^{n} x_k^{(j)}(\theta_k), 0, \ldots, 0\right) = f_k\left(\sum_{j=1}^{n} x_k^{(j)}(\theta_k)\right), \tag{3}$$

where $x_k^{(i)}$, $i = 1, 2 \ldots, n$ are doses of the same agent (with marker θ_k).

Besides the equalities (2) and (3), note also the following difference. For an ordinary function of many variables, given that all arguments are equal to the same value varying over a certain domain, we obtain a function of one variable. For the dose-response function in this case, the same multifactorial dependence is preserved, since the values of the arguments, although equal, remain tied to different markers.

However, marker notation for dose variables is not used in theoretical works (nor in the text below). Instead, a specific ordered sequence of agents is considered, and an agent is determined by its position in that sequence.

The following definition presents a formalized and more correct variant of the notion of zero interaction from (Berenbaum, 1985) Appendix 1.

Definition 1. *Let there be given n real variables* x_i, $i = 1, 2, \ldots, n$, $x_i \in [0; D_i]$ *and the function* $Y(x_1, \ldots, x_n)$, *defined on a product* $\prod_{i=1}^{n}[0; D_i]$. *Let* $f_i(x_i)$ *denote the function of one variable defined by the equality* $f_i(x_i) = Y(0, \ldots, 0, x_i, 0, \ldots, 0)$. *We will say that the arguments* $(x_1^0, \ldots, x_n^0)$ *participate in a zero (additive) interaction with respect to agent* x_k *for a given response level* $Y_0 = Y(x_1^0, \ldots, x_n^0)$, *if there exist values* $x_k^{(1)}, \ldots, x_k^{(n)}$, $x_k^{(j)} \in [0; D_k]$, $j = 1, \ldots, n$, *of the argument* x_k *such that the following equalities are satisfied*

$$f_k(x_k^{(j)}) = f_j(x_j^0),\ j = 1, 2, \ldots, n,\ x_k^{(k)} = x_k^0 \tag{4}$$

and

$$Y_0 = Y(x_1^0, \ldots, x_n^0) = Y\left(x_k^{(1)}, \ldots, x_k^{(j)}, \ldots, x_k^{(n)}\right) = f_k\left(\sum_{j=1}^{n} x_k^{(j)}\right) \tag{5}$$

Due to the equality (4), the values $x_k^{(j)}$ can be called *isoeffective* for the values $x_j^0, j = 1, \ldots, n$.

The Definition 1 states that zero interaction is not absence of interaction; rather, it is an interaction that could be effectively thought of as a joint action of the same agent taken in different doses. In other words, we consider the interaction of an agent with itself as a prototype of any zero interaction. Since in such an interaction of the same agent with itself, taken in different doses, the latter are summed up and, thus, such a joint action is equivalent to the single-factor action of this agent at a dose equal to the sum of doses, this type of joint action is also called *additive*.

Note that for the existence of isoeffective doses $x_k^{(j)}$ it is necessary to ensure that the ranges of the single-factor dose-response functions should be somehow consistent, for example, coincide. This condition will be satisfied automatically if the condition (6) of scale equivalence is met (this notion was introduced without a name in (Berenbaum, 1985)).

Definition 2. *Let us call the monotonic functions $f_i(x_i), i = 1, \ldots, n, x_i \in [0; D_i]$, of one variable scale equivalent if there is a monotonic function $g(x), x \geqslant 0$, and for each $i = 1, \ldots, n$ there exists $\lambda_i > 0$ such that we have the equality*

$$f_i(x_i) = g(\lambda_i x_i). \tag{6}$$

In toxicology, zero interaction of a set of toxic agents is understood as a property of the *entire* set of these agents. At the same time, the Definition 1 explicitly depends on the agent x_k. However, for the scale equivalent dose-response functions the notion of zero interaction does not depend on the agent chosen (see Theorem 3), i.e. it holds or does not hold for each agent simultaneously. Thus, in this case, it is correct to state that the property of zero interaction is inherent in the entire set of agents, but it is not true in the general case.

3. General Theorem on Zero Interaction Manifold

In this section, we explore the construction of a zero interaction manifold without the assumption (6) but under condition of the *same monotonicity* for all single-factor dose-response functions $f_i(x_i)$. This case will also be called *unidirectionality* of one-factor functions. Since the objective is to find an analytical relationship between the components of the point $(x_1^0, \ldots, x_n^0)$, satisfying the Definition 1 of zero interaction, then the corresponding assertions of this type will be called *theorems on zero interaction manifold*.

In the statement of the Theorem 1 below, $\mathcal{E}(f)$ denotes the range of the real function f.

Theorem 1. *Let there be n variables $x_1, \ldots, x_n$ defined on the intervals $[0; D_i], x_i \in [0; D_i], i = 1, 2, \ldots, n$ and a function $Y(x_1, \ldots, x_n)$ of these variables defined on $\prod_{i=1}^{n}[0; D_i]$. Let $f_i(x_i)$ denote a function $f_i(x_i) = Y(0, \ldots, 0, x_i, 0, \ldots, 0)$. We assume that all functions f_i are monotonic in same sense (all increasing or all decreasing). Let following statements hold:*

1. *for a certain value $Y_0 = Y(x_1^0, \ldots, x_n^0) \in \mathcal{E}(f_k)$ the condition of zero interaction is met for a given combination $(x_1^0, \ldots, x_n^0)$ and for k from the Definition 1;*
2. *one has the equality $\mathcal{E}(f_i) = \mathcal{E}(f_k)$, for any $i \in \{1, 2, \ldots, n\}$.*

Then for the components of the combination $(x_1^0, \ldots, x_n^0)$ we have the equality

$$\frac{1}{X_k} \sum_{j=1}^{n} f_k^{-1}\left(f_j(x_j^0)\right) = 1, \tag{7}$$

where X_k is an isoeffective dose of the argument x_k, satisfying the equality $f_k(X_k) = Y_0$, and f_k^{-1} is an inverse function.

Proof. Since the ranges of the functions $f_i(x_i)$ are the same set, then for any $j = 1, 2, \ldots, n$ there exists a value $x_k^{(j)}$ of the argument x_k (where k is an index relative to which the equality (5) is met) such that we have the equality

$$f_k(x_k^{(j)}) = f_j(x_j^0)$$

Since for any function $y = f_i(x_i)$ exists an inverse function $f_i^{-1}(y)$, then we have the equality

$$x_k^{(j)} = f_k^{-1}\left(f_j(x_j^0)\right), \quad \text{or} \quad x_j^0 = f_j^{-1}\left(f_k\left(x_k^{(j)}\right)\right) \tag{8}$$

Consequently, *from the point of view of observable characteristics*, manifested in the values of the arguments (doses of the factors) and the values of the functions f_i, Y (resulting effects), the vector $(x_1^0, \ldots, x_n^0)$ (the initial mixture of different substances) is indistinguishable from a vector consisting of n components of the same substance (namely x_k) taken in doses $x_k^{(j)}$, $j = 1, \ldots, n$ (sham combination). Indeed, in a "blind" experiment in which the experimenter does not know the formulas of the acting substances (their "markers"), knowing just the experimental doses of the individual substances and corresponding (isolated) effects, it is impossible to distinguish the initial mixture of different substances from a mixture of one substance, the components of which are taken in accordance with the equality (8).

Thus, the dose combinations $(x_1^0, \ldots, x_n^0)$ and $\left(x_k^{(1)}, \ldots, x_k^{(n)}\right)$ are equivalent in the sense of single-factor effects:

$$\left(f_1\left(x_1^0\right), \ldots, f_n\left(x_n^0\right)\right) = \left(f_k\left(x_k^{(1)}\right), \ldots, f_k\left(x_k^{(n)}\right)\right)$$

Since the doses were assumed to satisfy the Definition 1, we have the equality

$$Y\left(x_1^0, \ldots, x_n^0\right) = Y\left(x_k^{(1)}, \ldots, x_k^{(n)}\right)$$

Consequently, it follows from (1) and from the latter equality that

$$Y_0 = Y\left(x_1^0, \ldots, x_n^0\right) = f_k\left(\sum_{j=1}^{n} x_k^{(j)}\right)$$

Furthermore, there exists an isoeffective dose X_k corresponding to the response level Y_0 : $Y_0 = f_k(X_k)$. Thus, we have the equality

$$Y_0 = f_k(X_k) = f_k\left(\sum_{j=1}^{n} x_k^{(j)}\right)$$

Hence, due to the monotonicity of the function f_k, the equality follows

$$X_k = \sum_{j=1}^{n} x_k^{(j)},$$

which is equivalent to the equality (7), taking into account (8). □

It follows from the proof of the Theorem 1 that, strictly speaking, the dose combination that satisfies the zero interaction condition also satisfies the equation (7). Thus, it may be stated that the zero interaction manifold *is contained* in the manifold described by the equation (7), but, possibly, does not coincide with it. In fact, on the manifold (7) lie only those points for which the Definition 1 holds, as it follows from the next converse theorem to the Theorem 1.

Theorem 2. *Under Theorem 1 conditions, let the combination* $(x_1^0, \ldots, x_n^0)$ *satisfy the equation* (7). *Then for the point* $(x_1^0, \ldots, x_n^0)$ *the conditions* (4) *and* (5) *of the Definition 1 are met.*

Proof. Let the equation (7) be satisfied for the point $(x_1^0, \ldots, x_n^0)$. Let us denote the value of the multifactorial function Y at the point $(x_1^0, \ldots, x_n^0)$ as Y_0 : $Y(x_1^0, \ldots, x_n^0) = Y_0$. Then the values $x_k^{(j)}$ of the k-th agent defined by the equalities

$$x_k^{(j)} = f_k^{-1}\left(f_j(x_j)\right)$$

are obviously isoeffective for the dose $x_j^0, j = 1, 2, \ldots, n$. Hence we have the condition (4).

Since, by condition, X_k is an isoeffective single-factor dose of the k-th agent corresponding to value Y_0, then we have the equalities

$$Y(x_1^0, \ldots, x_n^0) = Y_0, \quad f_k(X_k) = Y_0$$

Hence,

$$Y_0 = Y\left(x_1^0, \ldots, x_n^0\right) = f_k\left(\sum_{j=1}^{n} f_k^{-1}\left(f_j(x_j^0)\right)\right) = f_k\left(\sum_{j=1}^{n} x_k^{(j)}\right) =$$

$$= Y\left(0, \ldots, 0, \sum_{j=1}^{n} x_k^{(j)}, 0, \ldots 0\right) = Y\left(x_k^{(1)}, \ldots, x_k^{(n)}\right)$$

Consequently, the condition (5) of the Definition 1 is met. □

Thus, the equation (7) is an analytical representation of those and only those points for which the Definition 1 holds. In other words, equation (7) specifies a set in the space $\prod_{i=1}^{n}[0; D_i]$ on which lie those and only those points for which the zero-interaction condition is satisfied (relative to variable x_k). From here, in particular, it follows that if the gradient of the function $f_k\left(\sum_{j=1}^{n} f_k^{-1}\left(f_j\left(x_j\right)\right)\right)$ is nondegenerate on the set of zero-interaction points, then this set is a smooth manifold of dimension $n-1$ in the n-dimensional space $\prod_{i=1}^{n}[0; D_i]$.

Let us present some corollaries from the Theorem 1. The equation (7) can be given the following symmetrical form.

Corollary 1. *Under conditions of the Theorem 1, the equation* (7) *may be presented as follows*

$$\sum_{j=1}^{n} \frac{f_k^{-1}\left(f_j(x_j^0)\right)}{f_k^{-1}\left(f_j(X_j)\right)} = 1, \tag{9}$$

where X_j *is an isoeffective dose of the agent* x_j, *satisfying the equality* $f_j\left(X_j\right) = Y_0$.

Proof. Indeed, according to the Definition of the isoeffective dose X_j, $j = 1, 2, \ldots, n$, corresponding to the response level Y_0, we have the equalities $f_j(X_j) = f_i(X_i) = Y_0$. Hence, $X_k = f_k^{-1}(Y_0) = f_k^{-1}(f_j(X_j))$, which gives the equality (9). □

The next statement shows that the Theorem 1 generalizes a similar theorem from (Berenbaum, 1985) proved under the condition of scale equivalence (6).

Corollary 2. *Under the conditions of the Theorem 1, and if the condition* (6) *of scale equivalence is met, the equation* (7) *reduces to the linear equation*

$$\sum_{i=1}^{n} \frac{x_i^0}{X_i} = 1, \tag{10}$$

where X_i is an isoeffective dose of the agent x_i, i.e. $f_i(X_i) = Y_0$, for all $i = 1, \ldots, n$.

Proof. It follows from the condition of scale equivalence (6) that if the equality $\gamma = f_j(x_j)$ holds for some $j \in \{1, \ldots, n\}$, then $x_j = \dfrac{g^{-1}(\gamma)}{\lambda_j}$. Hence, $f_k^{-1}\left(f_j(x_j)\right) = \dfrac{\lambda_j x_j}{\lambda_k}$, whence follows the equation (10). □

Thus, under the scale equivalence condition (6) the equation of zero interaction surface is linear and, consequently, determines a hyperplane in the space $\mathbb{R}^n$. In toxicology, pharmacology and theory of combined (joint) action, this linear equation is regarded to be a reference object for the characterization of other types of joint action. However, as can be seen from the proof, in deriving the equality (10) we relied heavily on the equality (6), without which the equality (10) cannot be obtained.

The importance of the zero interaction manifold is determined, among other things, by the fact that the type of joint action of n factors for a given dose combination will be determined by how this combination (a point in the n-dimensional space) is located relative to the zero interaction surface. Thus, the case of scale equivalent one-factor functions leads to the simplest zero-interaction surface, namely a hyperplane.

The next theorem shows that if the dose-response functions satisfy the scale equivalence condition (6), the notion of zero interaction does not depend on the agent and, thus, is correctly defined for the entire set of agents.

Theorem 3. *If under the conditions of the Theorem 1 the condition of scale equivalence* (6) *is met for one-factor dose-response functions, then the conditions* (4), (5) *of the Definition 1 are met for any $k = 1, 2, \ldots, n$.*

Proof. Let the Definition 1 be satisfied for the k-th agent and $m \in \{1, 2, \ldots, n\}$, $m \neq k$. Provided the scale equivalence condition (6) is satisfied, it follows from the quality $f_i(x_i) = f_j(x_j)$ that $\lambda_i x_i = \lambda_j x_j$.

Let $x_m^{(j)} = \dfrac{\lambda_k x_k^{(j)}}{\lambda_m}$, where $x_k^{(j)}$ are the isoeffective doses of the k-th agent satisfying the equality (4). Then $x_m^{(j)}$ are isoeffective doses of the m-th agent, i.e. we have the equality

$$f_m(x_m^{(j)}) = f_j(x_j) \quad \text{for any } j = 1, 2, \ldots, n$$

Indeed, allowing for the equality (6), we obtain

$$f_m(x_m^{(j)}) = g\left(\lambda_m x_m^{(j)}\right) = g\left(\lambda_k x_k^{(j)}\right) = f_k(x_k^{(j)}) = f_j(x_j)$$

Thus, the condition (4) is met for the m-th agent.

Since the Definition 1 is satisfied for the k-th agent, then the condition (5), is also satisfied for the m-th agent.

$$Y_0 = Y(x_1,\dots,x_n) = Y\left(x_k^{(1)},\dots,x_k^{(n)}\right) = f_k\left(\sum_{j=1}^{n} x_k^{(j)}\right) = g\left(\lambda_k \sum_{j=1}^{n} x_k^{(j)}\right) =$$
$$= g\left(\lambda_k \sum_{j=1}^{n} \frac{\lambda_m x_m^{(j)}}{\lambda_k}\right) = g\left(\lambda_m \sum_{j=1}^{n} x_m^{(j)}\right) = f_m\left(\sum_{j=1}^{n} x_m^{(j)}\right) = Y\left(x_m^{(1)},\dots,x_m^{(n)}\right),$$

which is what we intended to check. □

Moreover, it can be shown that the coincidence of zero interaction isoboles for different agents (at the same response level) is equivalent to the scale equivalence of one-factor functions.

The next lemma is obvious.

Lemma 1. *A set of monotonic positive functions* $\{f_1(x), f_2(x),\dots,f_n(x)\}$ *defined for* $x \geqslant 0$ *satisfies the scale equivalence condition* (6) *if and only if there exist a number* $j \in \{1,\dots,n\}$ *and a set* $\{\mu_1,\dots,\mu_n\}$ *of positive numbers such that the equality* $f_i(x) = f_j(\mu_i x)$ *holds for each* $i = 1,\dots,n$.

Theorem 4. *Let the conditions of Theorem 1 be satisfied and for each* $k \in \{1,\dots,n\}$ *all the zero interaction isoboles with respect to the k-th agent coincide. Then the functions* $f_1(x),\dots,f_n(x)$ *are scale equivalent, i.e. the equality* (6) *holds for these functions, and the zero interaction isobole is determined by equation* (10).

Proof. Let us fix a number $j \in \{1,\dots,n\}$. Without loss of generality, we can assume that all functions $f_i(x)$ equal to zero at $x = 0$: $f_i(0) = 0, i = 1,\dots,n$. Take a number $m \in \{1,\dots,n\}, m \neq j$, and consider zero interaction isoboles with respect to j-th and m-th agents (both corresponding to the same response value Y_0). By assumption they represent the same surface. From Corollary 1 it follows that these isoboles can be represented by equation (9). Then the following equations represent the same geometric set of points (in the space of dose combinations)

$$\frac{x_j}{X_j} + \frac{f_j^{-1}(f_m(x_m))}{f_j^{-1}(f_m(X_m))} + \sum_{i=1,i\neq j,m}^{n} \frac{f_j^{-1}(f_i(x_i))}{f_j^{-1}(f_i(X_i))} = 1$$
$$\frac{f_m^{-1}(f_j(x_1))}{f_m^{-1}(f_j(X_1))} + \frac{x_m}{X_m} + \sum_{i=1,i\neq j,m}^{n} \frac{f_m^{-1}(f_i(x_i))}{f_m^{-1}(f_i(X_i))} = 1$$

Let $x_i = 0$, for each $i \neq m$. Then we get the following equation

$$\frac{f_j^{-1}(f_m(x_m))}{f_j^{-1}(f_m(X_m))} = \frac{x_m}{X_m}$$

Hence,

$$\frac{f_j^{-1}(f_m(x_m))}{x_m} = \frac{f_j^{-1}(f_m(X_m))}{X_m} \Leftrightarrow \frac{f_j^{-1}(f_m(x_m))}{x_m} = \frac{X_j}{X_m} = \mu_m$$

Thus, $f_m(x_m) = f_j(\mu_m x_m)$, and Lemma 1 completes the proof. □

Consequently, in the general case, the zero interaction isoboles turn out to be different for different agents and will coincide if and only if their one-factor functions satisfy the scale equivalence condition (6). By virtue of Corollary 2 such an isobole will necessarily be linear.

When processing the results of biological and medical experiments in order to analyze a combined effect with the help of isoboles, it is common practice to construct an approximation of the dose-response function based on the available experimental data. *A priori* no additional conditions are imposed on these approximations apart from a certain form of analytical expression (e.g. a polinomial in x_i). The main objective of this stage of analysis is to find an expression that would represent the experimental data most accurately (in this or that sense). Consequently, one should not expect that the condition of scale equivalence (6) would be met in this case.

If we asssume from the outset that the multifactorial dose-response relationship is determined in such a way that the condition (6) is satisfied, then the quality of the appriximation of experimental data by such a function may turn out to be insufficient.

A trade-off would be the choice of a function as a candidate for approximating of a multifactorial dose-response relationship, which would have quite a lot of parameters for a good-quality fitting of the data and, at the same time, the condition (6) would be satisfied. For instance, this could be a function

$$\gamma = g\left(p_n(x_1, \ldots, x_n)\right), \qquad \text{where}$$
$$p_n(x_1, \ldots, x_n) = \sum_{i=1}^{n} b_i x_i + \sum_{i<j} b_{ij} x_i x_j + \cdots + b_{1\ldots n} x_1 \ldots x_n,$$

and $g(x)$ is a monotonic function on $\mathbb{R}$, $g(0) = 0, b_i > 0$ for all i. Of course, in the range of doses of interest for the researcher, not all of the conditions of the Theorem 2 would be met, but they may be satisfied in at least some region of the dose combinations, which would enable one to gain some insight into the type of joint action in this particular case.

4. Scale Equivalence and Linear Zero Interaction Manifold

The Theorem 1 shows that the analytical representation of the zero interaction equation (7) is, generally speaking, nonlinear. If the scale equivalence condition (6) holds, this equation becomes linear (for the acting doses x_i). The linearity of the zero interaction isobole equation is considered in toxicology/pharmacology/biology as a proven fact. However, as can be seen from the equations (7), (9), this conclusion is erroneous for arbitrary dose-response functions.

This also follows from the fact that if the concept of zero interaction (see the Definition 1) and the form of the equations (7), (9) are rigorously defined, then this concept and corresponding equations remain dependent on the agent with respect to which the Definition 1 is valid.

On the contrary, if the condition (6) is met, this greatly simplifies the situation and the following properties are true

- the ranges of the dose-response functions of the agents are the same;
- the isoeffective doses of the agents are proportional for any level of the effect,

which, in turn, guarantees

- the independence of the notion of zero interaction from the agent, i.e. if the Definition 1 holds for one agent, it would be met for other agents as well;
- the linearity of the equation defining the zero interaction manifold.

Remark 1. *In (Berenbaum, 1985) author argued against Loewe that the line of additivity (i.e. zero interaction line) may be other than a straight line for arbitrary dose-response functions. On the contrary, the author*

of (Berenbaum, 1985) believed that irrespective of the type of dose-response relationship, zero interaction is represented by a straight line (hyperplane) expressed by the equation (10)[1]*. According to the Theorem 2, this is indeed so if the condition* (6) *is met. However, without this assumption this conclusion is erroneous. In the general case, the zero interaction manifold is described by the equation* (7) *or* (9)*, which would not be linear for arbitrary functions of the dose-response relationship.*

Consider a simple example.

Example 1. *Let two agents* A_1 *and* A_2 *take their dose values on* $[0; 1]$*, and their single-factor dose-response relationships be given by*

$$f_1(x_1) = x_1 \qquad f_2(x_2) = x_2^2.$$

Since these functions do not appear to be related by the equation (6)*, the zero interaction equation for them is given by* (7) *(or, in symmetrical form, by* (9)*), which leads to the following zero interaction equations (provided the Definition 1 is met in relation to the first and second agents, repsectively)*

$$x_1 + x_2^2 = const, \quad for\ k = 1; \qquad \sqrt{x_1} + x_2 = const, \quad for\ k = 2.$$

The corresponding isoboles have a different form and depend on k (see Fig. 1)

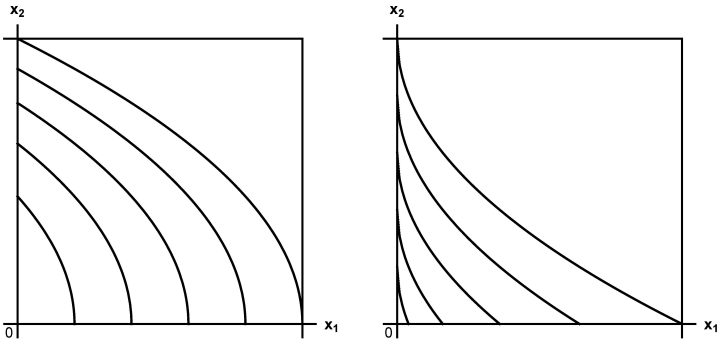

Figure 1. Non-linear zero interaction isoboles: left — with respect to the first agent; right — with respect to the second agent.

Consequently, for one-factor dose-response relationships not satisfying (6), the zero-interaction manifold may be nonlinear and be depending on which agent the zero interaction condition is met for. The assumption of linearity of the zero interaction isobole may lead in this case to an incorrect estimate of the type of combined action exhibited by such agents. In particular, if we thought that the zero-interaction isoboles were necessarily linear, then in the example on Fig. 1, we would have to say that these isoboles express a subadditive or superadditive joint action of the agents whereas in fact they are zero interaction curves (with respect to the corresponding agent).

5. Examples

[1]Citation Berenbaum, 1985, p. 418: "The type of dose-effect relation is immaterial; it may be the same or different for the various agents in the combination, and there is no requirement that the relations be expressible as simple algebraic functions, as in the cases described above." Or Berenbaum, 1985, p. 418: "It can be shown (Appendix 1) that, if the agents in a combination do not interact in producing the effect of the combination then, irrespective of dose-effect relations, the isobole for that effect satisfies the equation…" (the reference to (10) is given).

5.1 Some Response Function Models

The function representing the dependence of a response on given factors, as a rule, remains unknown. For gaining an insight into that dependence a researcher constructs an approximation of the response function based on available experimental data. In general, this method of constructing the approximation of a dose-response relationship presents the well-knonw technique of finding an adequate linear statistical model (Clarke, 2008; Janke & Tinsley, 2005; Khuri, 2010).

Note that the use of linear statistical models together with the concept of design of experiment is based on the response surface theory (Anderson & Whitcomb, 2016; Izelu *et al.,* 2013; Kappele, 2017; Myers *et al.,* 2016). Initially, the latter was proposed for finding such a set of parameters to describe the functioning of a system that would allow the optimal values of the target function to be achieved (with respect to a given criterion and allowing for preset restrictions). It was originally developed for applications in the chemical industry, but it is now used also in biological, clinical, and social sciences (Greco *et al.,* 1995; Khuri, 2001; Syracuse & Greco, 1986; Weinstein *et al.,* 1990).

The most common type of model in the response surface theory is polynomial models, and these are no higher than second order (see (Box & Draper, 2007; Khuri & Mukhopadhyay, 2010; Myers *et al.,* 2016)). These models are divided into three classes:

- first-order linear model, or *main effects model*

$$y = b_0 + \sum_{i=1}^{p} b_i x_i + \varepsilon \tag{11}$$

- linear model with cross terms, or *main effects model with interaction*

$$y = b_0 + \sum_{i=1}^{p} b_i x_i + \sum_{i,j=1,i<j}^{p} b_{ij} x_i x_j + \varepsilon \tag{12}$$

- quadratic model

$$y = b_0 + \sum_{i=1}^{p} b_i x_i + \sum_{i,j=1,i<j}^{p} b_{ij} x_i x_j + \sum_{i=1}^{p} b_{ii} x_i^2 + \varepsilon \tag{13}$$

In all cases, the variables x_i are independent factors, y is the response being studied, ε is error of the model.

The models (11)–(13) may also be used for describing the joint action of agents by the isobolographic method. However, the main-effects linear model (11), obviously, would always generate linear isoboles, which are traditionally interpreted as representing zero interaction. The presence of more complex types of joint action may be established only with the help of higher-order models, for instance, (12), (13). Note also that the correctness of conclusions on the type of joint action is directly dependent on the quality of the approximation by models (11), (12) or (13). Although these models may look relatively simple, they are widely used in various studies; in particular, they have been applied to tackle biological problems in (Chang *et al.,* 2017; Nazni & Gracia, 2014; Trinh & Kang, 2010).

5.2 Hyperbolic Model

To describe the joint action of two agents, a minimal model (12) is often used. Let us demonstrate that for this model, the condition (6) is satisfied, if the coefficients b_1, b_2 are of the same sign, and the conclusions about the type of joint action based on the assumption of the linearity of isobole and use of the model (6) are correct.

Obviously, in the model (12) for two variables

$$y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2$$

the intercept term b_0 may be omitted. Thus, we obtain the model

$$y = b_1x_1 + b_2x_2 + b_{12}x_1x_2$$

Hence, the single-factor dose-response functions are given by

$$f_1(x_1) = b_1x_1, \quad f_2(x_2) = b_2x_2$$

Therefore, if b_1, b_2 are of the same sign, they satisfy the equality (6), where $g(x) = x$, if b_1 and b_2 are positive, and $g(x) = -x$, if they are negative. In both cases $\lambda_i = |b_i|$.

5.3 Quadratic Model

Another model often used to describe two-factor dose-response relationships is a quadratic polynomial in two variables (Box & Draper, 2007; Myers *et al.,* 2016), i.e. the model (13) . In this model, one-factor functions are given by equalities

$$f_1(x_1) = b_0 + b_1x_1 + b_{11}x_1^2 \tag{14}$$

$$f_2(x_2) = b_0 + b_2x_2 + b_{22}x_2^2 \tag{15}$$

Hence, here the one-factor functions will be invertible only in a certain domain of the independent variables x_1, x_2. This means that for the quadratic model (13), the conditions of the Theorem 1 may not be satisfied. Moreover, even if the one-factor functions (14), (15) display the same monotonicity in the domain of experimental values, it is necessary to check whether their ranges of values coincide. If not, the domains of these functions need to be narrowed to ensure that the corresponding ranges coincide. One can check that the equality (6) for the functions (14),(15) will be satisfied if and only if the coefficients b_1, b_2 are of the same sign and the equality holds

$$\left(\frac{b_1}{b_2}\right)^2 = \frac{b_{11}}{b_{22}} \tag{16}$$

Thus, for the quadratic model (13), we cannot assume that the condition (6) for one-factor functions (14), (15) is always satisfied and, consequently, that the zero interaction isoboles are given by the equation (10).

However, in this case as well, there may exist dose intervals for each of the agents at which the functions (14), (15) have the same monotonicity and the same set of values on them. Then we can apply the Theorem 1, and the zero-interaction isobole equation is given by (7) or by (9).

5.4 A Practical Example

Consider an example from (Nazni & Gracia, 2014), where the effects of Refined Wheat Flour (x_1) and Barnyard Millet Bran (x_2) on the bread were explored. This experiment used a central composite design, in which x_1 varied from 70 to 100 g, and x_2 varied from 5 to 30 g, while in orthogonal encoding the variables changed from –1 to 1. The response y was represented by 10 different characteristics of bread, of which we only consider "Overall acceptability". To approximate the response, the model (13) was used. The model's determination coefficient was 0.88. The model equation is given by (in the orthogonal encoding of the independent variables)

$$y = 5.2 + 0.3x_1 - 0.91x_2 + 0.21x_1x_2 + 0.21x_1^2 - 0.25x_2^2$$

Since the Definition 1 and the Theorem 1 assume that the domains of the variables are of the form $[0; a]$, then we apply a linear transformation to the variables x_1, x_2 so that new variables are defined on the segment $[0; 1]$. We obtain the equation (17) (the old notation is kept for the independent variables)

$$y = 5.98 - 0.66x_1 - 1.24x_2 + 0.84x_1x_2 + 0.84x_1^2 - x_2^2, \tag{17}$$

where the variables x_1, x_2 take values on $[0, 1]$. The one-factor functions (14), (15) are then given by the equations

$$f_1(x_1) = 5.98 - 0.66x_1 + 0.84x_1^2 \tag{18}$$

$$f_2(x_2) = 5.98 + 1.24x_2 - x_2^2 \tag{19}$$

Since these functions do not satisfy the equality (16), the zero interaction isoboles will not be described by the equation (10). Moreover, according to the Definition 1, the shape of the zero interaction isobole will depend on which function the zero interaction condition is met for.

We should start by searching for intervals on which the functions $f_1(x_1)$ and $f_2(x_2)$ have same monotonicity and the same range of values. It is easy to check that on the intervals $x_1 \in [0, 0.3929]$,$x_2 \in [0, 0.09697]$ the functions f_1, f_2 demonstrate the same monotonicity (decrease), and have the same range of effects which is the segment $[5.85, 5.98]$. Besides, the range of the two-factor response function (17) is the segment $[5.75, 5.98]$ and contains the range of the functions $f_1(x_1), f_2(x_2)$. Thus, inverse functions $f_1^{-1}(y)$ and $f_2^{-1}(y)$ are defined on the segment $[5.85, 5.98]$, the equations for which are given by

$$f_1^{-1}(y) = 0.0119048\left(33 - 1.73205\sqrt{2800y - 16381}\right) \tag{20}$$

$$f_2^{-1}(y) = 0.02\left(-31 + \sqrt{15911 - 2500y}\right) \tag{21}$$

Let us take from the range $[5.85, 5.98]$ few values for which there are isoboles of the function (17) and hypothetical zero interaction isoboles given by the equation (7). Namely, we consider isoboles for the following values of effects 5.86, 5.885, 5.91, 5.935, 5.96. Considering the Definition 1 relative to each of the functions $f_1(x_1)$ and $f_2(x_2)$, we obtain the following equations for the corresponding zero interaction isobles

$$k = 1: \qquad x_1 + f_1^{-1}\left(f_2(x_2)\right) = const_1 \tag{22}$$

$$k = 2: \qquad f_2^{-1}\left(f_1(x_1)\right) + x_2 = const_2, \tag{23}$$

where $const_1$ takes values 0.286, 0.190, 0.126, 0.075, and 0.032, and $const_2$ is equal to 0.090, 0.072, 0.054, 0.035, 0.016.

The general location of the zero interaction isoboles for $k = 1$ and $k = 2$ is shown in Fig.2.

Thus, depending on which factor the Definition 1 is satisfied for, conclusions on the type of joint action will be different. The only case of an unambiguous conclusion is when the isoboles of the model (17) are located above or below the two isoboles in Fig. 2. However, this is not true for this case. For instance, the isobole of the model (17) corresponding to the level 5.86 is between the zero interaction isoboles corresponding to the same level of the effect (see Fig. 3).

It is clear that for the location of isoboles as in Fig. 3, the interpretation of the type of joint action would be ambiguous. In particular, for the response level 5.86 and the model (17), the joint action of the factors x_1, x_2 should be regarded as subadditive in relation to the first factor and superadditive in relation to the second one.

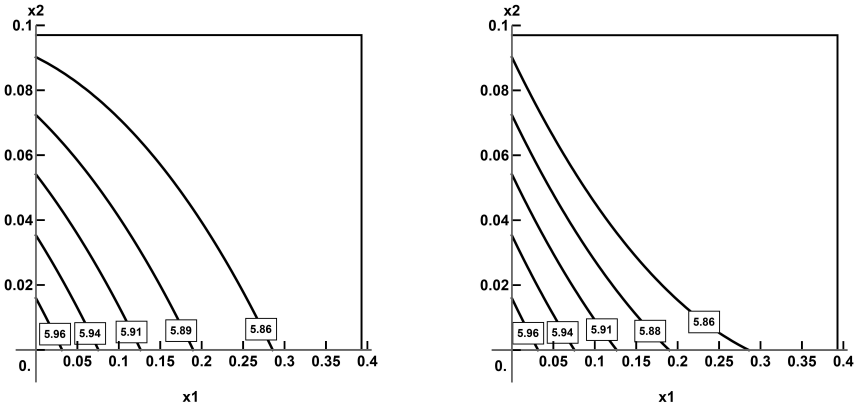

Figure 2. Zero interaction isoboles for the first (left) and second (right) factors for the equation (17). The numbers at each isobole are equal to a corresponding effect of the function (17).

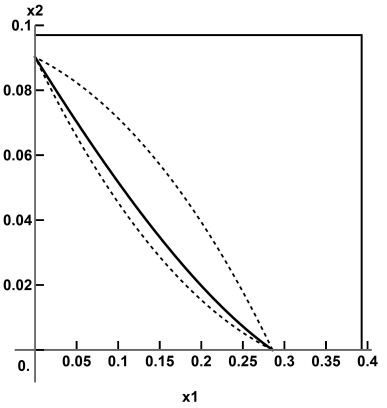

Figure 3. Zero interaction isboles for the level of the effect 5.86 relative to the first and second factors for the equation (17) (dashed lines) and the isobole of the same level for the model (17) (solid line).

5.5 Some Applications of the General Formalism

It is clear that the linearity of the zero interaction manifold proven for the assumption (6) significantly facilitates the analysis of the joint action of factors. Assuming that the zero interaction isobole is linear (i. e. it is a hyperplane in the space of dose combinations), a researcher may do not construct it explicitly and just find isoeffective single-factor doses of each agent doing this experimentally or with the help of an adequate model of the response function. Subsequent determination of the joint action type is carried out by placing the point corresponding to a given dose combination relative to the zero interaction hyperplane.

At the same time, from the Theorem 1 it follows that in the general case (without the condition (6)) the zero interaction isobole (7) has a more complex shape, being, in particular, nonlinear. In addition, it is necessary to take into account in relation to which agent we are considering Definition 1 and, accordingly, draw conclusions about the type of joint action depending on this specific agent. Thus, it is not always possible to draw a conclusion about the type of joint action without specifying in relation to which agent the zero interaction concept is being considered. Below we consider some constructions usually employed in the joint action analysis, and some their modifications in view of the equation (7).

A direct version of this analysis is based on estimating the value of *interaction index* $\mathbb{I}$, defined by

the equality

$$\sum_{i=1}^{n} \frac{x_i}{X_i} = \mathbb{I} \tag{24}$$

Comparing this sum with 1 (more-less-equal) determines the type of combined action for a given dose combination (subadditivity, superadditivity and additivity, respectively).

Taking into account the equation (7), it turns out that an analogue of the interaction coefficient $\mathbb{I}$ depends on the agent in relation to which we consider the Definition 1 and has the form

$$\frac{1}{X_k} \sum_{j=1}^{n} f_k^{-1}\left(f_j(x_j)\right) = \mathbb{I}_k$$

Subsequent analysis of the combined action in this case may be performed in the same manner as for the equation (24).

The linear equation (10) was used in a more sophisticated way in (Sühnel, 1992). Let the one-factor dose-response functions $f_i(x_i)$, $i = 1, \ldots, n$ be known (or adopted as acceptable approximations). Based on these functions and the interaction coefficient $\mathbb{I}$, a construction proposed in (Sühnel, 1992) for hypothetical response function for which the interaction coefficient at each point is equal to $\mathbb{I}$. To this end, an implicit equation for the effect γ as a function of the doses x_i and the parameter $\mathbb{I}$ is considered

$$\sum_{i=1}^{n} \frac{x_i}{f_i^{-1}(\gamma)} = \mathbb{I} \tag{25}$$

This equation determines a ruled surface, which usually is not a plane and for which an isobole of any response level has an interaction coefficient $\mathbb{I}$. In particular, for $\mathbb{I} = 1$ we obtain a response surface Y° for which any isobole is described by the equation (10). This surface (manifold) is called (Sühnel, 1992) *zero interaction response surface*.

For instance, let $f_i(x_i) = 1 - e^{-(\alpha_i x_i)^{\mu_i}}$, $i = 1, 2, \ldots, n$ (the dose-response function is a Weibull probability density function). It is not difficult to verify that these functions meet the scale equivalence condition (6), so that the zero interaction manifolds are hyperplanes.

From the equation (25), we obtain the following equation for the surface (25) with a given value $\mathbb{I}$

$$\sum_{i=1}^{n} \frac{\alpha_i x_i}{\left(-\ln\left(1-\gamma\right)\right)^{1/\mu_i}} = \mathbb{I} \tag{26}$$

If all $\mu_i = \mu$, then we can derive from the equation (26) the value of the effect γ as a function of the doses as follows

$$\gamma(x_1, \ldots, x_n) = 1 - \exp\left[-\left(\frac{1}{\mathbb{I}} \sum_{i=1}^{n} \alpha_i x_i\right)^{\mu}\right] \tag{27}$$

In particular, for the zero interaction surface (i.e. $\mathbb{I} = 1$) for $\mu = 1$, $n = 2$ we obtain from (27) a response function Y°, the shape of which is shown in Fig. 4.

It is interesting that for the parameters chosen, the function $Y^\circ(x_1, x_2)$ satisfies the equality

$$Y^\circ(x_1, x_2) = Y(x_1) + Y(x_2) - Y(x_1) \cdot Y(x_2),$$

where $Y(x_i) = f_i(x_i) = 1 - \exp\left(-\alpha_i x_i\right)$, $i = 1, 2$. Indeed,

$$\begin{aligned} &Y(x_1) + Y(x_2) - Y(x_1) \cdot Y(x_2) = 1 - \exp\left(-\alpha_1 x_1\right) + 1 - \exp\left(-\alpha_2 x_2\right) - \\ &- \left(1 - \exp\left(-\alpha_1 x_1\right)\right) \cdot \left(1 - \exp\left(-\alpha_2 x_2\right)\right) = 1 - \exp\left(-(\alpha_1 x_1 + \alpha_2 x_2)\right) = Y^\circ(x_1, x_2) \end{aligned}$$

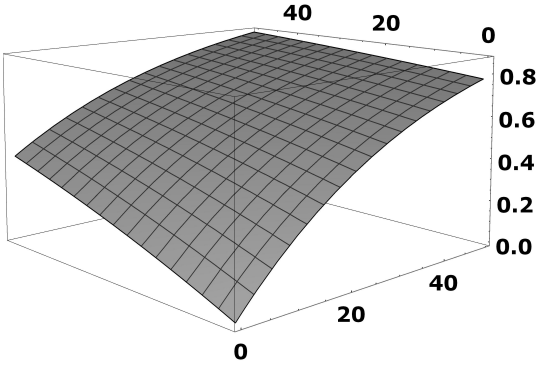

Figure 4. Zero interaction response surface Y° for $\mathbb{I} = 1$, $\mu = 1$, $\alpha_1 = 0.03$, $\alpha_1 = 0.01$ for the equation (27).

Thus, the hypothetical zero interaction response surface for dose-response functions defined by the Weibull distribution at $\mu = 1$ satisfies the Bliss independence condition (Bliss, 1939), demonstrating that there is a potential connection between these approaches to estimating the type of joint action. However, this relationship has not yet been studied in sufficient detail.

It is important to note that the zero interaction surface in the sense of (Sühnel, 1992) is a hypothetical response surface for which all isoboles (at any reasonable level of the effect) satisfy the equation (10). The term "zero interaction manifold" used above is a section of a multidimensional response surface by a (hype)plane of constant effect and it is not the same as the hypothetical zero interaction response surface in the sense used in (Sühnel, 1992).

As can be seen, in both approaches described above, the fundamental role is played by the linearity of the expression for defining the interaction coefficient $\mathbb{I}$, which, in turn, returns us to the theorem on the construction of zero interaction isoboles in the case of scale equivalence of one-factor dose-response functions (see the Corollary 2 and the equality (6)). If the equality (6) does not hold, the equation (7) (or its symmetrical variant (9)) should be used instead of (10). Thus, the equation for the hypothetical response surface with a given interaction coefficient $\mathbb{I}_k$ can be given by

$$\sum_{i=1}^{n} f_k^{-1}(f_i(x_i)) = \mathbb{I}_k f_k^{-1}(y),$$

where y is a given value of the effect, $X_k = f_k^{-1}(y)$. Clearly, this equation may be rewritten in explicit form describing the dependence of the effect y on the doses

$$y = f_k\left(\frac{1}{\mathbb{I}_k}\sum_{i=1}^{n} f_k^{-1}(f_i(x_i))\right) \tag{28}$$

For the two factor case surfaces (28) are given by the equations (for $k = 1$ and $k = 2$, respectively)

$$y = f_1\left(\frac{1}{\mathbb{I}_1}\left(x_1 + f_1^{-1}(f_2(x_2))\right)\right)$$
$$y = f_2\left(\frac{1}{\mathbb{I}_2}\left(f_2^{-1}(f_1(x_1)) + x_2\right)\right)$$

It can be easily verified that for the case of scale equivalence of one-factor dose-response functions

(6), the surface (28) is unique and is given by the equation

$$\gamma = g\left(\frac{1}{\mathbb{I}}\sum_{i=1}^{n}\lambda_i x_i\right)$$

In general, a review of the literature on combined action of factors shows that the linearity of zero interaction isoboles is not questioned and is used in all cases as a self-evident condition for representing zero interaction. However, there is some doubt as to whether this is always true (see, papers (Loewe, 1953; Loewe, 1957) and (Bosgra *et al.,* 2009)).

As follows from the Theorem 1, the zero interaction manifold in the general case is represented by a nonlinear equation. Moreover, this equation itself depends explicitly on which agent the zero interaction condition applies to. This makes it essential to take into account that it is not only the definition of zero interaction that depends on a specific agent, but the classification of combined action types (sub-, super-additivity, additivity) based on a corresponding equation will also depend on this agent.

6. Conclusions

Analysis of the joint action of factors based on the construction of isobolograms requires a strict definition of the zero interaction concept. Generally, this concept turns out to depend on a specific acting agent, and therefore the zero interaction isobole is also ambiguously defined. In addition, the zero interaction isobole can be a nonlinear manifold, which complicates the analysis of the combined action.

It was shown (Berenbaum, 1985) that if some condition on one-factor dose-response functions is met (this property is called the scale equivalence above in the article, see Definition 2), the isobole of zero interaction will indeed be a linear manifold (hyperplane, see equation(10)). However, in the general case it will be a nonlinear surface described by the equation (7).

It is important that the equation of zero interaction manifold and corresponding classification of the combined action types turn out to be dependent on the acting agent, which makes conclusions about the type of joint action also dependent on this agent. In particular, it may happen that the combined action in relation to one factor will be of a different type than in relation to another (see the example in Section 5.5). This poses an important problem for constructing adequate models of the response function for which the condition (6) is satisfied, since in this case the conclusion about the type of combined action will be unambiguous.

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Conflicts of Interest

The author declares no conflict of interest.

Author Contributions

The authors must include the CRediT- Contributor Role Taxonomy considering each author contribution for the paper, using the following roles (for more information, access https://credit.niso.org/):

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