CONSENSUS ON CONCEPTS AND TERMINOLOGY FOR COMBINED-ACTION ASSESSMENT: THE SAARISELKÄ AGREEMENT

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Abstract

A consensus was reached among the six authors regarding terminology and concepts for two-agent combined-actions. For the case in which both agents are effective individually, Loewe additivity is defined by the equation, $\frac{C_1}{EC_{X,1}} + \frac{C_2}{EC_{X,2}}$, in which C_1 and C_2 are the concentrations (or doses or intensities) of two agents (chemical or physical or abstract) in a mixture which elicits X% effect, and $EC_{X,1}$, $EC_{X,2}$ are the concentrations of each agent alone which would elicit X% effect. Bliss independence is defined by the equation, $fa_{12} = fa_1 + fa_2 - fa_1 fa_2$, in which fa_1, fa_2 and fa_{12} are the fractions of total possible effect affected by agent 1, 2 and the combination. For the cases in which the observed effects are more or less than predicted by these models, we propose the terms, Loewe synergism, Loewe antagonism, Bliss synergism and Bliss antagonism, respectively. When only one agent in a pair is effective alone, we propose inertism for the lack of influence of the second agent, synergism (without a leading adjective) for an increased effect caused by the second agent, and antagonism for the opposite case. When neither drug is effective alone, an effective combination is termed coalism. The relative merits of rival response surface models and model-fitting methodology is still unresolved. This "Saariselkä agreement" may form the nucleus for future standards in the field of combined-action assessment.

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Key words: Loewe additivity — Bliss independence — Loewe synergism — Loewe antagonism — Bliss synergism — Bliss antagonism — Inertism — Synergism — Antagonism — Coalism

Introduction

A group of six scientists with strong interests in the assessment of the joint effects of combinations of agents, met together for the Fifth International Conference on the Combined Effects of Environmental Factors, ICCEF'92, in Saariselkä, Finnish Lapland, September 6-10, 1992. The six scientists, from the fields of Pharmacology, Toxicology and Biometry, made presentations at the conference which underscored the controversies, disagreements and paradoxes endemic to the field of agent interactions. The group comprised a good representative sample of advocates of diametrically opposing views on many issues. These issues included: Which is the best null reference model for "no interaction", additivity (Loewe and Muischnek 1926) or independent effects (Bliss 1939)? Which is the central goal of combined-action studies, prediction of joint effects at agent concentrations not studied; i.e., both interpolation and extrapolation, or the discovery of biological mechanisms to explain observed joint action? Which is the ideal data analysis approach, 3-dimensional (3-D) parametric response surface fitting of the full data set, or 3-D nonparametric response surface fitting of the full data set, or 3-D parametric response surface fitting of the individual drug data with comparisons with the observed combination points, or 2-D concentration-effect curve fitting, or 2-D isobologram construction, or other approaches? A consensus on concepts, terminology, and data analysis approaches seemed remote. However, subsequent to the formal presentations and formal discussions, the group of six met privately, with the goal of reaching a working agreement on these issues. Surprisingly, a consensus on many issues was reached. This consensus, named the Saariselkä Agreement, is described below.

Table 1 lists the consensus terminology for the joint action of two agents. The foundation for this set of terms includes two empirical reference models for the situation in which each agent is effective alone, and in which each agent follows a monotonic, increasing or decreasing in the same direction, concentration-effect function. These models are Eq. 1, Loewe additivity (Loewe and Muischnek 1926), and Eq. 2, Bliss independence (Bliss 1939). The cases in which the observed effects are more or less than predicted by Eq. 1 or 2 are Loewe synergism, Loewe antagonism, Bliss synergism and Bliss antagonism, respectively. The use of the names, Loewe and Bliss, as adjectives, emphasizes the historical origin of the specific models, Eq. 1-2, and deemphasizes the mechanistic connotation of the terms additivity and independence. These two models have been the top candidates for a universal "no interaction" model in many diverse fields, and have been called by many other different names by many different authors. (Since the terms, "interaction" and "no interaction", can imply the presence or absence of a mechanistic association between the two agents, their use will also be limited in this article). A good history of these models and several others is provided by Kodell and Pounds (1991). Classic discussions which provided leads for unifying these two models were presented by Hewlett and Plackett (1959) and Ashford and Smith (1964). We have included both Loewe additivity and Bliss independence as reference models because each has some logical basis, and especially because each has its own coterie of staunch

Table 1. Consensus terminology for two-agent combined-action concepts

	both agents effective individually; Eq. 1 is the reference model	both agents effective individually; Eq. 2 is the reference model	only one agent effective individually	neither agent effective individually
combination effect greater than predicted	Loewe synergism	Bliss synergism	synergism	coalism
combination effect equal to prediction from reference model	**Loewe additivity**	**Bliss independence**	inertism	inertism
combination effect less than predicted	Loewe antagonism	Bliss antagonism	antagonism	

advocates who have skillfully and diligently defended their preferred model against repeated attacks. Each model looks at combined effects from a different viewpoint.

$$1 = \frac{C_1}{EC_{X,1}} + \frac{C_2}{EC_{X,2}} \qquad (1)$$

$$fa_{12} = fa_1 + fa_2 - fa_1 fa_2 \qquad (2)$$

In Eq. 1, and are the concentrations (or doses or intensities) of two agents (chemical or physical or abstract) in a mixture which elicits X% effect, and , are the concentrations of each agent alone which would elicit X% effect. It is assumed that EC_{X2} exist and are unique. (An alternative way of defining the variables in Eq. 1 is to have X represent a particular absolute effect level, instead of a particular relative X% level). The graphical use of this model is termed the isobologram approach, and was introduced by Loewe and Muischnek (1926). Many other algebraic, statistical and quasistatistical approaches consistent with the isobologram method have been developed; e.g., Berenbaum (1977, 1985, 1989). According to Eq. 1, the sham combination of an agent with itself will show Loewe additivity. In addition, when each of two different agents share the same "shape" for their concentration-effect curves, and differ only in relative potency, one agent can be thought of as merely a dilution of the other. This special case of Loewe additivity was termed simple similar action by Bliss (1939). However, Eq. 1 is commonly used in a more general manner, in which the concentration-effect curves for each agent may have different shapes. The different shapes are usually due to differences in "slope" parameters, but also may be due to differences in the type of concentration-effect functions. For this common general case, the theoretical basis for Eq. 1 is questionable. The arguments presented by Berenbaum (1989) for a theoretical validation of Loewe additivity are accepted by some researchers, but dismissed by many scientists as merely an exercise in circular logic. It may be that the occasional observation of Loewe additivity for combinations of two dissimilar agents is only fortuitous, and is devoid of any useful mechanistic implications. This is the chief criticism of Eq. 1 as a universal null reference standard. The defense is that, because of widespread usage, Eq. 1 is a useful standard for quantifying the intensity of interaction, and thereby facilitating predictions of effect at joint concentrations for which there is no data (interpolation and cautious extrapolation).

In Eq. 2, that for Bliss independence, fa_1, fa_2 and fa_{12} are the fractions of total possible effect affected by particular concentrations of agent 1, agent 2 and their combination, respectively. Essentially, Eq. 2 has the form of the common equation from the field of Probability, for the combination of probabilities for independent events (Rothman 1976; Zaider 1990). The theoretical basis for Eq. 2 is the intuitive idea of probababilistic independence; that two agents act in such a manner that neither one interferes with the other, but that each contributes to a common result (e.g., Webb 1963). Greater or smaller effects than expected for Bliss independently acting agents (or factors) represent enhanced or diminished relative effects in combination, compared with the effects of agents acting singly, after adjustment for the effect of the other agent. The main attack on Eq. 2, one which has been stressed by several authors (e.g., Berenbaum 1977), is that the sham combination of one drug with itself will often result in a conclusion of Bliss synergism or Bliss antagonism, depending upon the shape of the concentration-effect curve. However, this so called self-synergy or self-antagonism for an individual agent, which is counterintuitive to fans of the Loewe additivity reference model, is hailed by advocates of Eq. 2 as an important intrinsic property of many dose-response functions! (Note that Bliss independence cannot be defined for agents which do not demonstrate a maximum effect.)

It is clear that adherents of Loewe additivity and Bliss independence have heard all of the most compelling arguments for and against each model, and cannot be persuaded to switch allegiences. Thus, further progress with this debate is unlikely at the present time, and we propose that both models be tentatively accepted as legitimate empirical reference standards. This recommendation is made even though predictions of combined-effects based on each of the two rival reference models may be quite different, especially for very steep or very shallow concentration-effect curves. In addition, it must be emphasized that neither model unambiguously provides mechanistic explanations for the joint action of agents in complex systems, such as whole cells, single organisms, or populations of organisms. This is especially a challenge for the understanding of complex systems in which the observed response is quantal, such as dead/alive or success/failure. Links between combined-action phenomena and biochemical/physiological mechanisms have been derived only for relatively simple systems such as: two receptor agonists binding to the same receptor site (Ariëns et al. 1956); two enzyme inhibitors binding to the same enzyme (Webb 1963; Chou and Talalay 1981); and two inhibitors of simple metabolic pathways (Jackson 1991). In order for researchers to make mechanistic conclusions for a specific complex experimental system, the correspondence between empirical concepts such as Loewe synergism or Bliss antagonism, and theoretical mechanisms must be derived. This is a rich source for future research initiatives.

As shown in Table 1, when only the first agent in a pair is effective alone, we propose inertism for the case in which a second agent causes no change in the effect of the first agent, synergism (without a leading adjective) for an increased effect caused by the second agent, and antagonism for the opposite case. Leading adjectives can be omitted since inertism can be understood as a special case of both Loewe additivity and Bliss independence, synergism as a special case of both Loewe synergism and Bliss synergism, and antagonism as a special case of both Loewe antagonism and Bliss antagonism. When neither drug is effective alone, an ineffective combination may also be assigned the term inertism; whereas, an effective combination is termed coalism. (Note that although the action of a coalistic combination may be to increase or to decrease the measured response from the baseline; it is more common to define agent effects to increase with increasing agent intensity. Therefore, the lower righthand cell of the table is missing). For the cases in which more than two agents are present in a combination it is not fruitful to assign names to the higher order interactions.

A particular type of named interaction, such as Loewe synergism, may appropriately describe the entire 3-D concentration-effect surface. On the other hand, some agent combinations may demonstrate different types of combined-action at different local regions of the concentration-effect surface. Some researchers may even consider this situation to be a major tenet of the study of biologically-active agents. When this occurs, the combined-action terms in Table 1 can be used to describe well defined regions. However, it is important to differentiate true mosaics of different combined-action types from random statistical variation and/or artifacts caused by faulty data analysis methods.

For two-drug combinations, the characterization of the 3-D concentration-effect surface is often a reachable primary goal. When feasible, the fitting of response surface models to experimental data yields good summaries, with accompanying uncertainty measures. Prediction of joint effects in regions of the concentration-effect surface which do not contain data points (interpolation and extrapolation) is often a goal. Response surface methods can be parametric or nonparametric, can use a single 3-D equation to fit all data simultaneously, or can fit sets of 2-D concentration-effect curves or sets of 2-D isobols. After a 3-D response surface is fit to data, 2-D displays of both horizontal and vertical cuts through the surface are often useful to aid in the interpretation of the results. Deviations from either Loewe additivity or Bliss independence can be indicated with several types of algebraic, statistical and graphical approaches. The relative merits of rival response surface models and model-fitting methodology is still unresolved. This is another rich source for future research activities.

In conclusion, the Saariselkä Agreement represents a compromise among six widely differing viewpoints. The Agreement is a proposal, and is in no way binding upon any individual or group, but rather, may form the nucleus for future standards in the field of combined-action assessment. On a trial basis for the next two years, the authors will attempt to encourage the use of the terminology and concepts described in this document, and to accumulate feedback from the scientific community.

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