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From additivity to synergism — A modelling perspective

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Summary Interest in synergistic or antagonistic effects through mixture experiments has grown immensely over the past two decades, not the least within in pharmacology and toxicology. Several definitions of reference models exist; one commonly used reference model is concentration or dose addition, which assumes compounds, when administrated simultaneously, do not interfere with each other at the site of action. We focus on statistical modelling that allows evaluation of dose addition. We will describe several statistical approaches that are suitable for analysis mixture data where synergistic or antagonistic effects may be present. The statistical models are defined and explained and some of the approaches exemplified. Code in open-source software is provided.

1. Introduction

The interest in studying synergistic or antagonistic effects through mixture experiments has grown immensely over the past two decades, not the least within in pharmacology and toxicology. For instance, in pesticide sciences the quest for synergism is imminent, because synergism may lower the environmental load with pesticides. In pharmacology the quest for synergism is more of a mixed bag, because the synergism cuts both ways, in some particular examples it is wanted, in others it is not.

In the literature the definition of synergism and antagonism in relation to reference models is sometimes ambiguous as is the use of various reference models that often are considered contradictory. Two well-known reference models are dose addition and independent action [1]. Generally, dose addition assumes compounds, when administrated simultaneously, do not interfere with each other at the site of action, whereas independent action assumes that the compounds act independently of each other, i.e., at different site of action. In theory this makes it rather easy to choose a reference model, but compounds may have dose dependent secondary or even tertiary sites or modes of actions that blur the picture, particular when doing experiments involving living organism and not only for enzyme systems. Another issue is that for the metabolism of an organism virtually no site of action is not affected by an array of metabolic pathways.

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Due to the lack of knowledge on the part of researchers and scientists about which reference model to use, and the interest in patenting synergistic pesticide mixtures, some rather dramatic glitches have been published. The best known example, which caught a lot of attention, was how the mixtures of dieldrin, endosulfan or toxaphene were claimed to be 1000-fold potent as compared to the compounds administered alone on simple yeast estrogen system measuring estrogen-associated responses [2]. Firstly no laboratory could reproduce the results and secondly the paper used effect summation at ED50, a perhaps appealing method, which has limited value as pointed out by Kortenkamp and Altenburger [3]. Addition is limited to linear dose–response curves. However, generally dose–response curves are nonlinear with asymptotes. The paper by Arnold et al. [2] was retracted and resulted in numerous papers and disputes on the concept of synergy and reference models.

However, in fact there are two approaches: either use biological knowledge to select a combination model, or else use a testing framework to identify the model that best fits the data [4]. In order to be as unambiguous as possible we will focus on dose addition. Ideally dose addition is independent on the functional relationship as long as we are dealing with monotonically increasing or decreasing dose response curves or response-surfaces. Several specialized statistical approaches exists for evaluating dose addition [5–7]. Some approaches are quite simple and others remain challenging to apply. To our knowledge there has not been any attempt to compile such an overview of the very diverse collection of tools that exist for analyzing mixture data statistically. We present key ideas and approaches for detecting synergism. In particular, we will focus on statistical modelling. This means we will not pay much attention to the evaluation and interpretation of results.

In Section 2 we define the key concepts and introduce a number of statistical modelling approaches for quantifying synergy. Section 3 contains a few examples that illustrate the salient features of the different modelling approaches. Finally, in Section 4 we provide a perspective on alternative approaches in view of the advantages and shortcomings of the models presented.

2. Materials and methods

2.1. Additivity

Assume that a mixture compound defined as the combination of doses $x_1, \ldots, x_C$ of C compounds results in the average effect (or response) E. Following, Berenbaum [8] the mixture is said to be additive (for the specific effect E) if the following equation holds true:

$$\sum_{c=1}^{C} \frac{x_c}{f_c^{-1}(E)} = 1, \tag{1}$$

where $f_1, \ldots, f_C$ denote the assumed dose–response functions for the C individual compounds (more details below) and $f_1^{-1}(E), \ldots, f_C^{-1}(E)$ are doses of the compound resulting in an effect E (inverse regression). The effect E is the joint effect observed for the mixture. Thus each term in the sum in Eq. (1) is the dose of the compound in the mixture compound relative to dose of the individual compound that produce the same effect as the joint effect. This definition is also referred to as either dose addition or Loewe additivity [9]. There are a number of advantages in considering additivity in terms of doses rather than in terms of effects or responses [10]. However, one competing concept, which enjoys some popularity, is Bliss independence or independent action. We refer to Greco [5] and Tallarida [1] for discussions on how they differ.

Under additivity there is dose equivalence upon standardization by the individual compounds corresponding effective doses. If the C compounds are used in proportions $p_1, \ldots, p_C$, which sum to 1, the additivity equation may be written as follows:

$$\sum_{c=1}^{C} \frac{p_c\, f_{\text{mix}}^{-1}(E)}{f_c^{-1}(E)} = 1, \tag{2}$$

where f_{mix} denotes the assumed dose–response function for the mixture compound (using the total dose). Rearranging terms results in the equation:

$$f_{\text{mix}}^{-1}(E) = \frac{1}{\sum_{c=1}^{C}(p_c)/(\,f_c^{-1}(E))} \tag{3}$$

showing that if additivity is assumed the dose–response curve for the mixture is entirely determined by the dose–response curves for the individual compounds. Often the average effect considered is taken to result from a specified effective dose level (see below for binary mixtures). Also, the ratios on the left-hand side in Eq. (1) are sometimes called toxic units, indicating the relative contribution to toxicity of the individual compounds in the mixture compound.

The above definition is general and does not impose any constraint regarding how the dose–response data are analyzed statistically, except that usually some kind of regression model is used as is implicitly indicated by the inverse regression quantities in the above definitions. However, the regression curves are often assumed to be monotonically decreasing or increasing.

Below, we exemplify concepts specifically for binary mixtures as they are commonly used in practice.

2.1.1. Binary and ternary mixtures

For binary mixtures with only two chemicals ($C = 2$) the restricted hyperplane is simply a line segment in the first quadrant of the coordinate system, as shown in Fig. 1. So the binary mixture of two compounds A and B, say, is defined as the mixture $100p_A$% of A and $100p_B$% of B for some p_A, p_B ranging between 0 (individual B) to 1 (individual A) such that $p_A + p_B = 1$.

For effective dose levels resulting in a 50% inhibition (denoted by ED50_A and ED50_B) Eq. (1) simplifies to:

$$\frac{x_A}{\text{ED50}_A} + \frac{x_B}{\text{ED50}_B} = 1 \tag{4}$$

Likewise Eq. (3) may be written as:

$$\text{ED50}_{\text{mixture}} = \left(\frac{p_A}{\text{ED50}_A} + \frac{p_B}{\text{ED50}_B}\right)^{-1}. \tag{5}$$

For any other effective dose level (e.g., ED10 and ED20) similar equation may be obtained. Note that under the assumption of dose addition, Eq. (5) provides a means for predicting effective doses for any mixture compound based solely on the effective doses of the individual compounds [11].