

Statistical Aspects of Chemical Mixtures

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ABSTRACT

This paper reviews the considerable statistical research that has been performed in an attempt to characterize the joint action of two or more chemicals from both a statistical modelling and an underlying biological mechanism point of view. Particular attention is paid to the traditional descriptions of joint toxic action, i.e. independent joint action, similar joint action, and synergistic action. The more generalized biological classification developed by Hewlett and Plackett and by Ashford and Cobby are also considered. When chemicals are not interactive but affect similar sites, it seems that some knowledge of the likely toxicity of a mixture can be gained from the individual toxicities of its constituents. However, more biologically complex situations clearly require an understanding of the toxic mechanisms of the mixture before such projections can be made with any degree of confidence.

1 INTRODUCTION

Toxicology is primarily concerned with the study of a variety of individual chemicals in an experimental system. How does one estimate the response in that experimental system for any mixture of these chemicals? This is the fundamental question central to the understanding of the joint action of chemicals in toxicology. The traditional description of these and other issues from a statistical standpoint is given in the classic book by Finney (1971).

According to Finney, the first systematic discussion of the issues in the context of quantal response was given by Bliss (1939). In this work Bliss described three types of joint toxic action: (1) independent joint action, (2) similar joint action, and (3) synergistic action. In the first of these types, the chemicals are considered to act independently and to have different modes of action. As a result, the toxicity of a mixture can be predicted statistically from the responses to the individual chemicals. The second category, similar joint action, is one in which the chemicals produce a similar effect in the experimental organism and one chemical can be substituted at a constant proportion of the other with predictable effects. This means that the toxicity of the mixture can be predicted from the knowledge of the

behaviour of the individual chemicals. Finally, the third category, synergistic action, includes all cases not describable as either independent joint action or similar action. When there is synergy one cannot statistically predict effects without a basic understanding of the mechanisms of the toxicity of the individual chemicals involved.

2 SIMPLE INDEPENDENT ACTION

The typical example to illustrate simple independent action is the case of two chemicals which produce effects in very different ways although the observed response is the same. This would be the case when we are considering, for example, death of the organism with the only recorded data being either the death or survival of each experimental unit.

Suppose for chemical 1 the probability of death after an administration of dose d_1 is represented by $P_1(d_1)$ and for chemical 2 by $P_2(d_2)$. Further, we take the probability of death of the organism after administration of both dose d_1 of chemical 1 and dose d_2 of chemical 2 to be $P(d_1, d_2)$. Assuming simple independent action between chemical 1 and chemical 2, we find that the probability of death after administration of doses d_1 and d_2 of each of the two chemicals is described by

$$P(d_1, d_2) = P_1(d_1) + P_2(d_2) + P_1(d_1)P_2(d_2) \quad (1)$$

This simple relationship is problematical when one considers the usual probit model for the dose-response relationship of the individual chemicals. For example, suppose P_1 and P_2 are both cumulative normal distributions. Then, even if the individual probit regression lines are parallel or are, in fact, equal, the distribution $P(d_1, d_2)$ will not follow a normal distribution in terms of total dose $d_1 + d_2$. This has been discussed in detail by Finney. He describes the type of curve that one obtains for a fixed mixture of the individual chemicals when the response to each chemical follows a linear probit relationship.

Wahrendorf and Brown (1980) have also examined the relationship described by Equation (1). They point out that if independent action is the true state of nature and Equation (1) is therefore correct, one is then confronted with a pair of inequalities on the probability of effect from the joint administration of the chemicals, namely:

$$\max \{P_1(d_1), P_2(d_2)\} \leq P(d_1, d_2) \leq \min \{1, P_1(d_1) + P_2(d_2)\} \quad (2)$$

The authors further describe the use of bootstrap estimation techniques for purposes of determining if either of these two inequalities is violated. In such an instance, one would conclude that the two chemicals do not follow an independent joint action model.

Continuing their investigation of simple independent action, Wahrendorf *et al.* (1981) considered the generalization of simple quantal data in relation to the

problem of animal survival studies. By using the probability of no effect, which is defined to be $Q = 1 - P$, they rewrote the defining Equation (1) as

$$Q_{00}Q_{11} = Q_{10}Q_{01} \quad (3)$$

where

$$Q_{01} = 1 - P_1(d_1), Q_{10} = 1 - P_2(d_2), Q_{11} = 1 - P(d_1, d_2)$$

and

$$Q_{00} = 1 - P \text{ (spontaneous occurrence)}$$

By changing the notation they were able to include the term for the spontaneous occurrence of an effect, namely $1 - Q_{00}$. Equation (3) was then re-expressed by Wahrendorf as a log-linear model, namely:

$$\log Q_{ij} = \mu + \alpha_i + \beta_j + \delta_{ij} \quad (i = 0, 1; j = 0, 1) \quad (4)$$

where δ_{ij} is 0.

Now the hypothesis of simple independent action which implies Equations (1) and (3) becomes

$$H_0: \delta_{11} = 0 \quad (5)$$

The critical quantity is the maximum likelihood estimate of δ_{11} and in particular its estimated variance. Wahrendorf considered the design problem for detecting possible interactions using the criteria of minimizing the variance of the maximum likelihood estimate of the interaction term in the log-normal model. Using this strategy and a few assumptions, he developed some appropriate designs of experiments for testing H_0 . Continuing this problem to the chronic animal studies for carcinogenesis, the authors considered the analogous hypothesis to that given in Equation (3). In this case the Q terms are replaced by the survival functions which are now functions of time t . The hypothesis of no interaction can be written in terms of cumulative cancer hazard functions as

$$H_0: G_{00}(t) + G_{11}(t) - G_{10}(t) - G_{01}(t) = 0 \quad \text{for all } t \quad (6)$$

where $G_{ij}(t) = -\log Q_{ij}(t)$.

By making proportional hazard assumptions, the problem then reduces to parameters which are independent of time. Essentially, this hazard assumption relates the survival functions to the Weibull distribution. Wahrendorf has shown that if the proportional hazard model is appropriate, they can test the simple independent action hypothesis in a long-term carcinogenesis study of two chemicals.

3 SIMILAR JOINT ACTION

When two chemicals have similar modes of action on the experimental subject, they often show parallel regression lines of probits of response on a log-dose

scale. When this is the case, the classic measure is that of relative potency, which is simply the ratio of equally effective doses. This ratio will be constant at all levels of response because the regression lines are parallel.

Suppose a mixture contains the amount Z_1 of chemical 1 and Z_2 of chemical 2. The chemicals show simple similar action if the response is equal to what would be produced by a dose of $Z_1 + \rho Z_2$ of the first chemical alone for some constant ρ . Here ρ denotes the relative potency of the two chemicals.

A few equations from Finney (1971) are needed to describe these relationships. For chemical 1 assume that the probit response Y_1 can be expressed in terms of a dose Z as

$$Y_1 = \alpha_1 + \beta \log Z \quad (7)$$

and for chemical 2

$$Y_2 = \alpha_2 + \beta \log Z \quad (8)$$

The potency of chemical 2 relative to chemical 1 is defined by

$$\log \rho = (\alpha_2 - \alpha_1) / \beta \quad (9)$$

For a mixture of the amount Z_1 of chemical 1 and Z_2 of chemical 2 it follows that the response can be given as

$$Y = \alpha_1 + \beta \log (Z_1 + \rho Z_2) \quad (10)$$

Further, if we consider Z to be the total dose in which the proportions of chemical 1 and chemical 2 are given by π_1 and π_2 , we can then write Equation (10) as

$$Y = \alpha_1 + \beta \log (\pi_1 + \rho \pi_2) + \beta \log Z \quad (11)$$

Equations (7), (8), and (11) produce an important relationship relating the response for the mixture to the responses for the individual component chemicals in the mixture. If, for example, LD_{50} is the dose that we are interested in, it follows from these three equations that

$$\frac{1}{\text{mixture } LD_{50}} = \frac{\pi_1}{LD_{50} \text{ for chemical 1}} + \frac{\pi_2}{LD_{50} \text{ for chemical 2}} \quad (12)$$

where π_i is the proportion of chemical i in the mixture.

The relationship expressed in Equation (12) is valid for other dose points, such as the LD_{10} . It can also be generalized to more than two chemicals as shown in the following relationship:

$$\frac{1}{\text{mixture } ED_x} = \sum_{i=1}^n \frac{\pi_i}{ED_x \text{ for chemical } i} \quad (13)$$

where $\sum_{i=1}^n \pi_i = 1$, and x is the proportion responding. In fact, Equation (13) holds

under similar joint action, even when the probit model does not describe the dose response.

This gives us a method for estimating the effect of the mixture based on the properties of the individual chemicals in the mixture as long as they all follow the simple similar action model.

Smyth *et al.* (1969) carried out a study in which 27 industrial solvents were tested for toxicity in rats with the LD₅₀ being the response of interest. In this large study the 27 chemicals were compared by pairwise testing. In the 350 pairs of chemical tests they conducted, the authors found that the harmonic mean formula given in Equation (12) satisfactorily predicted toxicity for a large proportion of the pairs of chemicals. For those pairs deviating from this relationship, the effects seemed to go in both directions; that is, they either underestimated or overestimated the LD₅₀ of the mixture. From the Smyth study one may therefore conclude that for the end-point of LD₅₀ and the type of organic chemicals considered, a simple similar action model reasonably predicts the effects of the two component mixtures based upon the effects of the individual chemicals.

4 BIOMATHEMATICAL MODELS

In a series of papers, Hewlett and Plackett (1959, 1961, 1964; Plackett and Hewlett, 1967) developed a more generalized biological classification for the various types of joint action. Also, they constructed mathematical models which described the joint action of two chemicals or drugs. The biological classification they considered (Hewlett and Plackett, 1959) was one in which joint action was considered to be either similar or dissimilar according to whether or not the sites of action of the two chemicals were identical. Further, they considered interactive or non-interactive action depending on whether or not one chemical influenced the biological action of the other. They described the two-by-two classification by means of the following simple table:

		<i>Site of primary action</i>	
		Similar	Dissimilar
<i>Chemical influence on the biological action of one chemical by the other</i>	Non-interactive	Simple similar	Independent
	Interactive	Complex similar	Dependent

This two-by-two layout indicates four types of chemical action. The first two listed under non-interactive, namely simple similar action and independent action, have been previously discussed. One of the main features of the two-by-two classification by Hewlett and Plackett is the category of dependent action. Other investigators have generally considered the broad category of dissimilar

action to be independent. The authors, however, indicate examples of dependent action at dissimilar primary action sites. They in fact believe that, under the classification of dissimilar primary action, the dependent case will be more commonplace than the independent one. They predicate this hypothesis upon the idea that 'an organism is at least largely a coordinated whole.'

In developing mathematical models to describe joint action, Hewlett and Plackett considered threshold responses in which it is assumed that individual tolerances in the population will have a frequency distribution. Explicitly, they assumed that there is a dose Z^* such that if the administered dose Z exceeds Z^* then the response occurs.

One can reparameterize this for the effective dose at the site of action and consider the ratio of the transformed Z to the transformed Z^* and define this to be the variable U . We then find that the subject will respond at administered dose Z if the variable U exceeds one. If two chemicals are administered in doses Z_1 and Z_2 simultaneously, then the probability that the individual does not respond is the probability that

$$Q = P[U_1 \leq 1, U_2 \leq 1] \quad (14)$$

when responses are produced independently. For the case of similar joint action we can consider the sum of the fractions of the tolerances such that

$$Q = P[U_1 + U_2 \leq 1] \quad (15)$$

Hewlett and Plackett generalize these two cases by considering a parameter λ such that

$$Q = P[U_1^{1/\lambda} + U_2^{1/\lambda} \leq 1] \text{ for } 0 < \lambda \leq 1 \quad (16)$$

where for λ tending to 0 we obtain Equation (14) and for $\lambda = 1$ Equation (15). From this generalization we find that there would be a range of models among the non-interactive joint action category, varying from the independent case to the simple similar action case. Using normal theory in terms of log-dose and also instituting a correlation between the probit responses of the two chemicals, a fairly complicated mathematical structure is produced. For the simple similar action case, the Hewlett and Plackett model does provide a generalization, i.e. the slope of the response curves need not be parallel. In particular, if there is complete, positive correlation between tolerances one may write

$$e^{(X_1 - X)/\beta_1} + e^{(X_2 - X)/\beta_2} = 1 \quad (17)$$

where $X_i = \alpha_i + \beta_i \log Z_i$ and X represents the mixture response.

For the special case of $\rho_1 = \rho_2$ we see that Equation (17) is satisfied by Equations (7)–(10). In pharmacology, two drugs satisfying the relationship in Equation (17) are described as having additive action. It can also be shown that Equation (13) is still valid for the case of unequal response slopes β_i .

Ashford and Cobby (1974) have developed a system of models for joint action of drugs. Their approach differs from that of Hewlett and Plackett in that they consider the action of a drug at a site in terms of standard receptor theory. The physiological effect of a mixture of drugs or chemicals is then stated in terms of the occupancy of receptor sites at various sites of action in the organism. At a given site for a single chemical with an association constant a and concentration Z the proportion of occupied receptors is given by

$$P = \frac{aZ}{1 + aZ} \quad (18)$$

This relationship follows in accordance with the 'law of mass action'. The relationship extends to k drugs competing for the same receptor sites by the expression

$$P_i = \frac{a_i Z_i}{1 + \sum_{j=1}^k a_j Z_j} \quad (19)$$

where a_j is the 'association constant' for drug j , and Z_j is the concentration at the site of action.

It is assumed by the authors that the occupation of a receptor by a chemical changes the level of activity associated with the receptor by a factor α . Thus, at the site of action the level of activity is changed by a factor

$$(1 - P) + \alpha P = v(Z) = \frac{1 + \alpha Z}{1 + aZ} \quad (20)$$

This can be generalized to several chemicals:

$$v(Z) = \frac{1 + \sum \alpha_i a_i Z_i}{1 + \sum a_i Z_i} \quad (21)$$

After the application of the drug at dose Z to the site of action, it is assumed that the system is expressed as

$$y = f\{v(Z)\} \quad (22)$$

where f is a monotonic function of v .

If the response of the system is affected by the drug or drugs at more than one site, then $v(Z)$ is simply written as the product of the $v(Z)$ terms in Equation (21) at each site. We therefore assume a multiplicative effect by the action at separate sites. The authors then examine various models by constructing isobols, which are graphs of the loci of dosage values Z_i corresponding to a constant value of the response Y . With regard to the classification of joint action, the authors consider cases where synergism and antagonism are the result of joint action at a single site by competition for receptor sites of the chemicals involved in the mixture.

5 CONCLUSION

Considerable research has been conducted on the statistical aspects of joint action of drugs. The research has centred both on statistical models and on the biological classification of the joint action. One observes that, without some understanding of the mechanisms involved for a mixture of chemicals, one is not on very firm footing in terms of predicting the response of the individual chemical components.

Several authors, including Siemiatycki and Thomas (1981) and Hamilton (1982), have discussed the relationship between interaction of biological mechanisms and statements of interaction or synergism on the basis of statistical or public health measures. For example, if the response of a mixture of two chemicals is more than additive, from a public health viewpoint synergism is occurring; hence there is interaction involved with the two chemicals. However, from a biological standpoint, if one observes that one chemical is involved at one site in the toxicological process and the second chemical at a separate site, such as in the Ashford and Cobby approach, one could have multiplicative effects, hence synergism in the statistical sense. Both authors used multistage models of carcinogenesis to illustrate their points. They assumed that the various stages of the carcinogenesis model would correspond to separate sites of action as in the work of Hewlett and Plackett and of Ashford and Cobby. They observed that when two chemicals act at the same site they may act independently of one another and produce an overall additive effect, which implies a lack of synergism. On the other hand, the chemicals may be biologically independent and non-interactive at separate stages or sites, but may produce a multiplicative effect and hence synergism from a public health standpoint.

Among the various models, one at least has available the relationship given by Equation (13) where it is assumed that the chemicals are not interactive but affect similar sites and therefore come under the classification of simple similar action. This expression might be considered to be a rough approximation for estimating toxicity based upon knowledge of the toxicity of the individual chemicals. To proceed beyond this point numerically would require knowledge of the mechanisms of toxicity of the individual components of the mixture and also the pharmacological aspects of the chemicals used in combination. Therefore, as with many other issues in toxicology, one must have a knowledge of mechanism in order to predict effects outside the experimental range. Otherwise, one is forced to resort to estimates based upon what many consider to be fairly simplistic assumptions.

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