ANALYSIS OF HEALTH EFFECTS CAUSED BY MULTIPLE INSULTS

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ABSTRACT

A method is presented for the analysis of the risk of health effects caused by a combination of insults. The approach is entirely phenomenological and has no built-in restrictions. Also, interactions between the effects of various toxicants are treated in a general manner. The only restrictions arise from the finite set of functions relating exposure parameters and the risk of health effects. As an example, the incidences of oral and esophageal cancer in man are analyzed as a function of alcohol and tobacco consumption. The properties of the solutions obtained are discussed, together with conclusions about the processes involved in the etiology of these cancers.

KEY WORDS: Multiple Insults, Synergism, Antagonism, Data Analysis.

INTRODUCTION

In the environment, no toxicant can act on an organism all by itself, there is always a mixture of many agents acting in combination. In order to study the health effects of multiple insults, the investigation of exposures to only one and two toxicants provides the basic information needed for a theoretical approach. At present, experiments involving two toxicants are being planned with increasing frequency both in chemical and in radiological toxicology, because the presence of other toxic agents can enhance or diminish the effects of a toxicant and yield results which are significantly different from those expected for an additivity of damages.

Most of the data available involve the exposure to a dose of either toxic agent alone, followed by an exposure to the combination of the doses. A simple comparison then shows whether the effects are at, above or below additivity (Reif, 1984). However, a measurement at a single dose combination does not allow a detailed study of the interaction between the effects of the two toxicants. For such an analysis, more elaborate data sets are needed. At present, sufficiently large sets are available only from epidemiological studies (Tuyns et al., 1977; Walter, 1980; Whittemore and McMillan, 1983) but corresponding experiments both in vitro and in vivo are being planned by many experimenters.

In the absence of sufficient experimental data, a multitude of mathematical forms can be constructed for the interaction of two toxicants. It is, therefore, important to approach the analysis without preconceived notions as to the nature of the interaction and to rely on a phenomenological approach to reach conclusions which are, as far as

possible, subject only to the requirements of the data set. In addition, the first priority is to find the dominant ones among the many contributions possible, neglecting for the time being those of lesser importance.

It is the purpose of this study, to introduce a formalism which is completely phenomenological and has as few built-in restrictions as possible, and to use it in the analysis of epidemiological data to demonstrate the viability of the method.

SYNERGISMS, COERGISMS AND ANTAGONISMS

Combination of Risks

Risk are probabilities and the risks due to a combination of insults must, therefore, combine probabilities appropriately. Thus, the risk of a health effect due either to agent 1 or agent 2 is given by the sum of the two probabilities minus their overlap. The overlap is zero if and only if the effects caused by the two toxicants are mutually exclusive; it is equal to their product if the two probabilities are independent of each other; otherwise more complex forms apply. If more than two causes with probabilities r_i, are possible, the general expression for the combined risk of toxicants with independent actions is given by

$$r_{ind} = 1 - \prod_{i=1}^{n} (1 - r_i). \qquad (1)$$

Experimentally, it is often found, however, that the actions of several toxicants are not independent of each other, but that there are interactions between the effects of different insults. In that case, a risk higher or lower than r_{ind} is found. An experimental value R for the combined risk which is characterized by

$$R \begin{cases} > \\ = \\ < \end{cases} r_{ind}, \text{ is said to indicate } \begin{matrix} \text{Synergism} \\ \text{Coergism,} \\ \text{Antagonism} \end{matrix} \qquad (2)$$

respectively. For small risks, that is, for $r_i << 1$, the higher order products of the risks r_i are much smaller still, and the criterion for independent action on the right-hand side reduces approximately to the sum of the risks. This sum of risks is often - but incorrectly - used as a general criterion for the existence of synergisms or antagonisms. It is for this reason that the condition for equality in relation (2) is not labeled with the generally inappropriate term 'additivity', but with the broader term 'coergism'.

Combined Risks in the Presence of Interactions

If interactions are possible, the difference in inequality (2) can be assigned to interaction terms of second or higher order which describe the interaction between the effects of two or more toxic agents, respectively. The most general expression for the total risk of a mixture of n toxicants is thus

$$R = 1 - \prod_{i=1}^{n} (1 - r_i) + \sum_{i=1}^{n} \sum_{j=i+1}^{n} r_{ij} + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \sum_{k=j+1}^{n} r_{ijk} + \ldots. \qquad (3)$$

The second term on the right-hand side is the source of the overlap terms of up to nth order between the risks r_i, if they are assumed to be independent. The third and fourth terms describe the interaction between the effects of two and three toxicants, respectively. This basic structure of eq. (3) is determined by the requirement that it must reduce

to the marginal risk r_i if all exposures but the one to agent i are set equal to zero. Similarly, the interaction terms $r_{ij...k}$ must be zero if any of the exposures to agents i, j,...,k is zero.

For further discussions, it will become necessary to assume explicit cause-effect relationships for the marginal risks r_i. These may assume a variety of mathematical forms, but it will be assumed here that

a) the relevant exposure quantity is the accumulated dose D_i of the toxicant, and that

b) the dose-effect relationship is given by a power function of the dose D_i, that is, by

$$r_i = a_i D_i^{m_1} . \tag{4}$$

Whereas these assumptions will influence the detailed form of the results given here, the methods employed are general and can be used to analyze data involving dose-effect relationships which have several terms or which involve exposure quantities other than dose.

In view of the basic requirements for the interaction term $r_{12...m}$ between m toxicants, its most simple possible form is the product of all doses D_i. The next higher level of complexity is a product of powers n_j of the doses D_j.

$$r_{12...m} = a_{12...m} \prod_{j=1}^{m} (D_j)^{n_j}, \tag{5}$$

where the parameter $a_{12...m}$ is determined from experimental data. This form is appropriate for simultaneous exposures and, under certain conditions, for some consecutive exposures. If the interaction depends in any way on the sequence of some of the exposures, then a mathematical form has to be chosen which is asymmetric in the relevant doses.

Explicit Formula for Two Agents and Background

For two agents and a health effect with a background risk r_o, the most general form for the combined risk is

$$R = r_o + r_1 + r_2 + r_{12} - \{ Q \}, \tag{6}$$

where the overlap Q for independent action is defined by

$$Q = r_o r_1 + r_o r_2 + r_1 r_2 - r_o r_1 r_2. \tag{7}$$

Here, the marginal risks and the interaction term are potentially of the same order, whereas the overlap terms in Q are at least quadratic in the marginal risks r_i. Thus, for small values of the risks, the overlap Q can often be neglected.

In terms of the exposure parameters, the combined risk R is then given by

$$R = a_o + a_1 D_1^m + a_2 D_2^n + a_{12} D_1^p D_2^q, \tag{8}$$

The representation of the risks in which eqs. (6) and (8) are given is called the absolute risk model. It assumes that the marginal risks r_o, r_1, r_2 and the interaction term r_{12} all have different dependences on age, sex, and some lifestyle parameters. Its 4 parameters a_i and a_{ij} are the absolute risk coefficients, to be determined from experimental data.

If it is assumed that all dependences except those on exposure are the same, the background risk r_o can be factored out, resulting in the relative risk model, which thus assumes that the combined risk is proportional to the background risk

$$R = r_o[1 + f_1 + f_2 + f_{12} - \{ Q' \}], \tag{9}$$

where

$$Q' = r_o[f_1 + f_2 + f_1 f_2 - r_o f_1 f_2]. \tag{10}$$

Here, the symbols f_i and f_{ij} denote the fractional excess risks for the two agents and their interaction, respectively, and Q' is the relative overlap for independent action of the toxicants. The quantity in square brackets is called the relative risk and is independent of age, sex and some lifestyle parameters.

In the low-dose region, if the relative overlap Q' is very small and can be neglected, the combined risk in terms of the doses D_i is

$$R = a_o[1 + b_1 D_1^m + b_2 D_2^n + b_{12} D_1^p D_2^q], \tag{11}$$

with the relative risk coefficients b_i and b_{ij}. Thus, if the relative risk R/a_o is fitted to an experimental data set, 3 parameters have to be determined.

ANALYSIS OF EPIDEMIOLOGICAL DATA

Analytical Approach

The characteristics of some biological processes which lead to the endpoint under consideration manifest themselves not only in the dose-effect relations of the marginal risks, but are particularly evident in the algebraic form of the interaction terms. The main purpose of the analysis is, therefore, an attempt to extract the mathematical essence of the information contained in the data set.

Geometrically speaking, the fitting of a data set by eqs. (8) or (11) is equivalent to fitting a risk surface to the data points in the 3-dimensional space defined by the two dose axes D_1 and D_2 and the risk axis R (For an aid to visualization, see Fig. 3). The approach used here is to determine the surface with the most simple algebraic structure which gives an acceptable fit to the data. Thus, the first trial functions are planes, then surfaces are used which are curved in one or more dimensions.

Algebraically speaking, this means that at first all linear and quadratic forms of both doses are tested in all combinations. There are 44 such trial functions, characterized by their exponents in eq. (11) which are combined in the symbol (mn pq). In addition, the interference term is given a special form, found already in previous evaluations of synergisms (Reif, 1984; Whittemore and McMillan, 1983). This results in a separable form of eq. (11), that is, in a product of factors each related to only one toxicant

$$R = a_o[1 + b_1 D_1^m][1 + b_2 D_2^n]. \tag{12}$$

For linear and quadratic forms of the marginal risks, there are 4 such functions. Separable functions will be denoted by the symbol (mn*mn), whereas the absence of a marginal risk or of an interference term is characterized by (m0 pq) and (mn 00), respectively.

Analysis of Two Data Sets

There are two data sets that relate the incidence of cancer of the upper gastro-intestinal tract to the consumption of alcohol and tobacco. The first is a study of Tuyns et al. (1977) of esophageal cancer in the province Ille-et-Villaine of France; the second an investigation of the incidence of oral cancer in the United States (Walter, 1980). A full discussion of the details of both data sets and of their analyses are given elsewhere (Seiler, 1985), only results and conclusions will be summarized here.

The 48 trial functions were tested not only for the quality of their fit to the data but also for the stability of these fits for moderate changes in the doses. Both the fitted surfaces and the values of the parameters fitted proved to be surprisingly stable, although the sum of weighted least-squares per data point sometimes changed considerably. The best fits were determined on the basis of goodness of fit and the requirement that the coefficients obtained be clearly nonzero.

Four trial functions consistently yielded the best fits, and two of these also fulfilled the criteria for nonzero coefficients. They are the functions characterized by (22 00) and (12*12). The first assumes independent action of the toxicants and quadratic marginal risks; the second assumes a synergistic, but separable risk function and relative risks linear in tobacco and quadratic in alcohol consumption. It should be noted that whereas most of the 48 trial functions have 3 free parameters, the best fits were obtained by two more restrictive functions with only 2 parameters (Figs. 1 and 2).

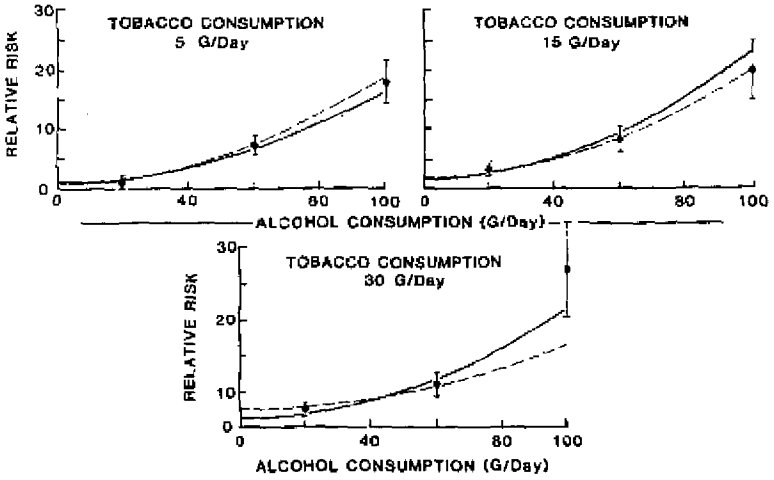

Fig. 1. Relative risk of esophageal cancer as a function of alcohol and tobacco consumption. The data points are those of Tuyns et al. (1977) and the solid and dashed lines are fits for the trial functions (12*12) and (22 00), respectively.

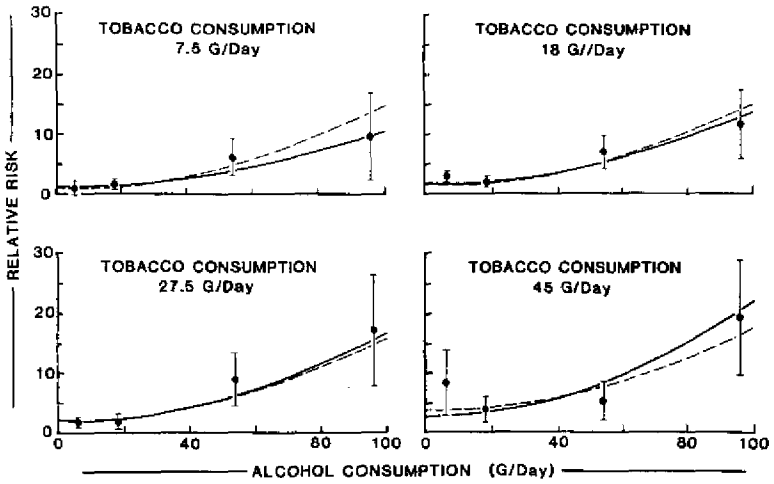

Fig. 2. Relative risk of oral cancer as a function of alcohol and tobacco consumption. The data points are those of Walter (1980) and the solid and dashed lines are fits for the functions (12*12) and (22 90), respectively.

For both oral and esophageal cancer, the best trial functions were the same, and even more important, the relative risk coefficients were the same, resulting in the same risk surface (Fig. 3). This implies that cancer of the oral cavity and the esophagus due to the consumption of alcohol and tobacco have a similar, if not the same etiology. Also, it allows to combine both organs into one critical organ for these toxicants.

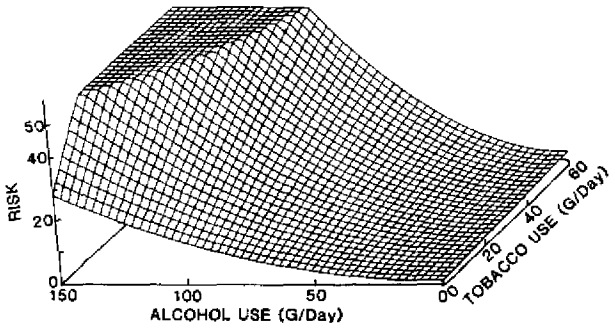

Fig. 3. Surface for the relative risk for both oral and esophageal cancer as a function of alcohol and tobacco consumption. In order to show the curvature of the surface it has been cut off at a relative risk of 50.

Mathematically, there are no valid reasons to prefer one of the two best solutions over the other. Biologically, however, there are reasons for a preference. In the complex multi-stage evolution from a normal cell to a cancerous cell, there are likely to be several steps where interaction can occur and result in a nonzero interaction term. On the other hand, the existence of two totally independent multi-stage pathways leading to the same cancer, one induced by alcohol and the other by tobacco seems considerably less plausible. For this biological reason, the solution (22 00) is considered the less likely one.

The separable structure of the more likely solution (12*12) requires that the synergistic interaction is of a kind which allows the risk enhancement due to one toxicant to be totally independent of the enhancement due to the other agent. Thus, although there is a dependence in a statistical sense, there must be independence in the mechanism of the interaction. This requirement restricts the type of processes that mechanistic models may use to describe the pathogenesis of oral and esophageal cancer by alcohol and tobacco.

Finally, the separability of the most probable solution and the equality of the risk surfaces for both cancers can lead to the formulation of some hypotheses with regard to the histologogy of the cancers or particular processes in their etiology. These hypotheses could then be verified in future experiments.

DISCUSSION

The purpose of this study was to introduce a formalism for the analysis of data from experimental studies involving a combination of insults. The method involves a minimum of a priori assumptions and attempts to distill the mathematical essence of the way in which biochemical processes influence the dependence of health effects on exposure.

The implementation of this method which is discussed here in detail uses an approach common in other fields by assuming that, until more is known about the processes studied, only the dominant contributions are of interest and that only the simplest solutions which are compatible with the data should be determined. More complex approaches to eqs. (3) to (5) can be devised easily, once additional knowledge on the health effects is available.

The application of this methodology to two totally independent sets of epidemiological data on cancers with different locations in the upper gastro-intestinal tract leads to several important results, demonstrating the capability to determine the numerical values of risk coefficients, and to discriminate between different algebraic structures of the interaction term. From these results, inferences can drawn and hypotheses formulated, which lead to new, clearly defined experimental questions.

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