QUANTAL HEALTH EFFECTS FOR A COMBINATION OF SEVERAL TOXIC AGENTS

Abstract — Quantal health effects caused by the combined action of a number of toxic agents are modeled using the information available for each toxicant acting in isolation. Two basic models are used; one assumes no interaction, the other postulates a separable kind of interaction, in which each agent contributes an enhancement factor independent of all other agents. These two models provide yardsticks by which to measure synergisms and antagonisms in the interaction between the effects of toxic agents. Equations are given in approximations for small and large values of the risk.

PRINCIPAL INVESTIGATORS

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The evaluation of the potential effect of a particular toxic agent in man's environment must also consider the simultaneous exposure to a multitude of other toxicants. A general formalism for combination effects for two or more agents and a background incidence has been given earlier (1983-84 Annual Report, LMF-113, pp. 323-332). Consideration is restricted to "nonsignature" quantal health effects, that is, to health effects for which the most important characteristics are their occurrence or nonoccurrence and that they show no differences that would allow an identification of the etiological agent. Two examples of this type of effects are mutation and cancer induction.

In the analysis of data on the incidence of quantal health effects such as cancer, an independent action model will provide a yardstick against which to measure the influence of synergistic or antagonistic interactions between the effects of the toxic agents present. This model depends exclusively on data for the marginal risks. The second model used here is based on the observation that in every analysis of data involving a combination of insults,[1-3] a separable risk function is a viable solution. A separable risk function has the property that it can be separated into factors that depend solely on the exposure to one agent and can thus also be calculated from marginal risk data. Similar to the independent action model, a separable interaction model provides a simple measure for actual interactions.

It is the purpose of this paper to present formulae, in terms of exposure parameters, for these two models, to assess their properties and their use as standards for occurrence and magnitude of interactions, as well as discuss their predictive power.

INTERACTION MODELS FOR COMBINED EFFECTS

Combined Effects of K Toxic Agents

There are many ways of defining the basic quantity "risk", usually depending on the area of application. One of the more versatile general characterizations defines risk as a triplet that consists of a particular scenario S, its probability P and its consequence C.[4] To distinguish between the same consequence C, for instance lung cancer, caused by exposure to K different toxic agents used by themselves (different scenarios S_k), the symbol r_k will be used for the marginal risks.

For quantal health effects, the general formula for the risk, r_{ind}, due to the independent action of K toxicants leading to the same "nonsignature" health effect, is given by the general formula for the OR-combination of K independent probabilities

$$r_{ind} = 1 - \prod_{k=0}^{K} (1 - r_k) \ , \qquad (1)$$

where the symbol r_k denotes the marginal risk for toxicant k acting in isolation and r_0 is the background risk. Written as a sum of terms of increasing degree, Equation 1 yields

$$r_{ind} = \sum_{k=0}^{K} r_k + \sum_{k=0}^{K-1} \sum_{i=k+1}^{K} (-)\, r_k\, r_i + \cdots$$

$$+ \sum_{k=0}^{K-m} \cdots \sum_{i=l+1}^{K-1} \sum_{j=i+1}^{K} (-)^m\, r_k \ldots r_i\, r_j + \cdots + (-)^K \prod_{k=0}^{K} r_k \ . \qquad (2)$$

This can also be written as the sum of the probabilities minus the probablistic overlap Q, defined by

$$r_{ind} = \sum_{k=0}^{K} r_k - \{Q\} \ . \qquad (3)$$

Often Q is very small and can be neglected. The independent action risk is then equal to the sum of all the risks.

For the case of significant interactions between the effects of the K toxic agents, a number of interaction terms of various ranks have to be added:

$$r = r_{ind} + \sum_{k=1}^{K-1} \sum_{j=k+1}^{K} r_{kj} + \sum_{k=1}^{K-2} \sum_{j=k+1}^{K-1} \sum_{i=k+2}^{K} r_{kji} + \cdots + r_{123\ldots K} \ . \qquad (4)$$

The interaction terms must have an algebraic structure that leads to the correct lower form of Equation 4 if one or more of the toxic agents are used with a zero dose. As an example, setting the exposure to toxicant k to zero must result in the correct expression for K-1 toxic agents. At the lowest level, this requirement must yield the background risk if all toxicant exposures are set to zero.

For the simplest possible expressions for interaction terms, this yields the expressions

$$r_{ij\ldots Q} = \prod_{q=1}^{Q} F_q \ , \text{ with } Q \leq K \ , \qquad (5)$$

where the factors F_q depend exclusively on the exposure parameters to toxicant q.

Parametrization of Risks and Interaction Terms

If the accumulated dose D_k for a particular toxic agent is the relevant parameter connecting exposure and health effect, then the marginal risk r_k can be written as

$$r_k = a_k\, D_k^{m_k} \ . \qquad (6)$$

Here, the assumption is made that for small values of r_k one particular power of D_k makes the largest contribution to the risk, the other contributions being much smaller. Thus, Equation 6 contains only the dominant term.

Similarly, if only the dominant interaction term is selected, its simplest possible form is

$$r_{12\ldots Q} = a_{12\ldots q} \prod_{q=1}^{Q} D_q^{p_q} \quad . \tag{7}$$

This holds for both synergistic and antagonistic terms.

For large risks, the direct parametrization in terms of powers of dose needs to be replaced by a more appropriate one that takes into account that risks cannot be larger than unity. A popular form is the hazard function $H(D_k)$, connected to the risk r_k by

$$r_k = 1 - e^{-H(D_k)} = 1 - e^{-a_k D_k^{m_k}} \quad . \tag{8}$$

For small values of the hazard function $H(D_k) << 1$, a series expansion yields a first term equal to the right-hand side of Equation 6. Thus, for small values, the hazard function converges toward the risk function.

MODELS FOR DIFFERENT INTERACTIONS

Independent Action Model

For small risks and a background incidence of $r_0 = a_0$, the marginal risk of agent k is

$$r_k = a_0 + a_k D_k^{m_k} \quad , \tag{9}$$

assuming that there is one dominant contribution to the marginal risk, and that the overlap between background and excess risk is practically zero. Equation 3 then yields for the independent action risk of a combination of K toxic agents,

$$r_{ind} = a_0 + \sum_{k=1}^{K} a_k D_k^{m_k} - \{ Q \} \quad . \tag{10}$$

In this manner of presenting risks, called the absolute risk formulation, the excess risk due to the action of the agents is assumed to provide a term that is additive to the background risk. In the relative risk formulation,

$$r_{ind} = a_0 \left[1 + \sum_{k=1}^{K} b_k D_k^{m_k} - \{ \frac{Q}{a_0} \} \right] , \tag{11}$$

with

$$b_k \equiv \frac{a_k}{a_0} \quad . \tag{12}$$

The total risk is thus assumed to differ by an enhancement factor from a_0, i.e., the excess risk is proportional to the background risk.

For large values of the risk, that is, for risks for which the condition $r_k << 1$ does not hold, Equation 1 can be rewritten using Equation 8;

$$r_{ind} = 1 - \exp\left[\sum_{k=0}^{K} H_k\right],$$
$$= 1 - \exp\left[\sum_{k=0}^{K} a_k D_k^{m_k}\right]. \qquad (13)$$

Here, there is no overlap to be subtracted, because independent action leads directly to the sum of hazard functions. Any additional terms in the exponent result in either synergism or antagonism. This simple form is an attractive feature of the hazard function formalism.

Separable Interaction Model

The separable interaction model derives its name from the fact that the risk function can be separated into a product of factors, each describing the action of one toxic agent. There are no nonlinear cross-terms within these factors, and in this mechanistic - not in a statistical - sense, the actions of the toxicants are independent. The difference between independence in a statistical and a mechanistic sense is that the former refers to the entire risk function, whereas *the latter refers only to each individual factor.*

Mathematically, the separable risk function is best written in the relative risk formulation. The marginal relative risks are then

$$R_k = \frac{r_k}{a_0} = 1 + f_k = 1 + b_k D_k^{m_k}, \qquad (14)$$

where f_k is the relative excess risk. The combined risk is then

$$r = a_0 \prod_{k=1}^{K} R_k = a_0 \prod_{k=1}^{K} (1 + f_k),$$
$$= a_0 \prod_{k=1}^{K} (1 + b_k D_k^{m_k}). \qquad (15)$$

for low values of the risk, that is for $a_0 R_k << 1$. *For higher values, the hazard function* approach yields an equivalent formulation,

$$r = 1 - \exp\left[-a_0 \prod_{k=1}^{K} (1 + b_k D_k^{m_k})\right], \qquad (16)$$

again derived from the requirement that for zero exposure to any agent, the correct equation for K-1 *agents should obtain, and at low values of the total hazard,* Equation 15 should result.

PROPERTIES OF TWO MODELS

Interpretation of the Algebraic Model Structure

If the independent action risk of Equations 10 or 11 is found in a data analysis, this means that the effects of one agent cannot influence the action of any other agent. In a multistep process such as carcinogenesis, a single sequence of steps cannot generally fulfill this requirement. There have to be either several independent sequences of steps, indicated by intervals and dots in Figure 1, leading to the same effect (Fig. 1A), or then a set of independent subsequences that join additively at some stage and form a single subsequence (Fig. 1B).

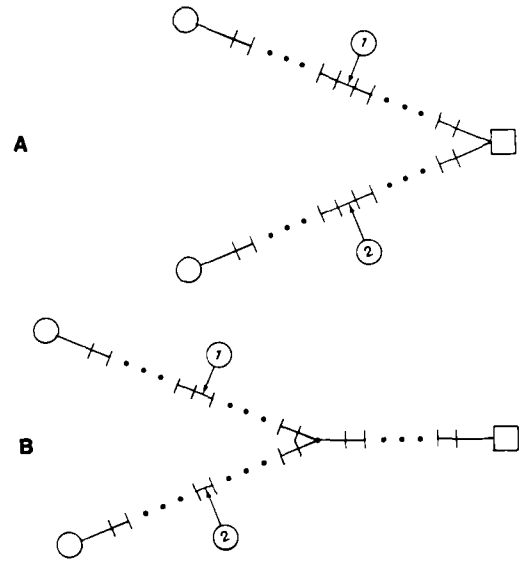

Figure 1. Schematics for independent action of two toxic agents: (A) completely independent sequences to the same health effect; (B) partly independent sequences requiring linear superposition of effects at the junction. The dots denote other stages, not influenced by the toxicants.

A separable risk function demands the mechanistic independence discussed above. If a multistep process is postulated, composed of a single sequence of steps, these limitations require that two or more agents cannot attack the same step directly and influence its probability appreciably. For the two or three direct-acting toxic agents in Figure 2A, this means that they have to enhance the probability of different steps in the process.

If two toxic agents act indirectly, for example by influencing the production of a third agent which then acts on one particular stage (Fig. 2B), then the enhancement must arise from different steps in that process. The mixture of a direct- and an indirect-acting agent in Figure 2C may also lead to a separable form as long as agents 1 and 2 attack different branches of that process. In this manner, the outcome of an analysis may be incorporated as an explicit or implicit assumption in a model using a separable form for the risk function.

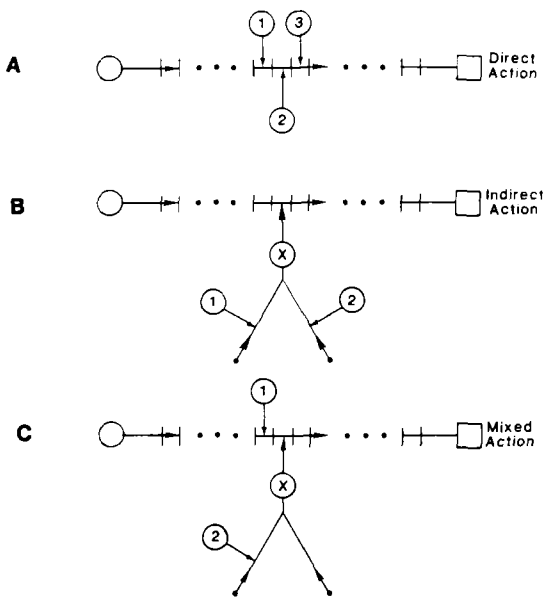

Figure 2. Schematics for separable interaction of two or three toxic agents: (A) direct acting agents, each enhancing a different stage; (B) indirectly acting agents, each independently enhancing the probability of the reaction producing the active metabolite; (C) a mixture of a direct and an indirect acting agent. The dots denote other stages, not influenced by the toxicants.

Predictive Power of These Models

The predictive power of these models is difficult to assess. On the one hand, four out of the four cases of epidemiological data analyzed[1-3] show compatibility with the two models, the separable interaction model being slightly preferred; on the other hand, four cases is a small number. Here, the new two-stage carcinogenesis models, which have been very successful in fitting age- and dose-dependence of some cancers, may give additional insights.[6-8]

The two-stage Moolgavkar-Venzon-Knudson model is shown schematically in Figure 3. C_1 denotes susceptible normal cells, C_2 transformed intermediate cells, and C_3 mutated cancerous cells. The cell transformation rates are denoted by $m_i(x,t) = m_i[\dot{d}_{m,i}(t),t]$: i is either 1 or 2; x is the exposure rate, here characterized by the dose rate $\dot{d}_{m,i}(t)$, which is relevant to cell type i and transformation to type i+1 as a function of time; and, t is the elapsed time. Analogously, birth and death rates of cell type i are denoted by the symbols $\alpha_i[\dot{d}_{\alpha,i}(t),t]$ and $\beta_i[\dot{d}_{\beta,i}(t),t]$, respectively. For the net growth rate of a cell population, the symbol $g_i[\dot{d}_{g,i}(t),t] = \alpha_i[\dot{d}_{\alpha,i}(t),t] - \beta_i[\dot{d}_{\beta,i}(t),t]$, is used. Note that the dose rates are potentially different for each effect of a toxic agent and may thus be used to describe the effects of combinations of different insults.

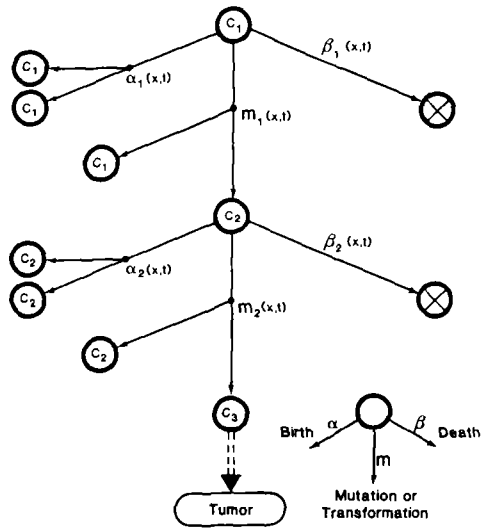

Figure 3. Schematic of the Moolgavkar-Venzon-Knudson model of carcinogenesis. Cells at C_1 are normal susceptible cells, those at C_2 are transformed to intermediate cells, and those at C_3 are mutated to carcinogenic cells. The quantities $m_i(x,t)$ are the transformation rates of cells C_i under exposure x at time t, whereas the quantities $\alpha_i(x,t)$ and $\beta_i(x,t)$ are the birth and death rates of these cells, respectively.

For small risks of a tumor at age τ, due to an exposure E to a combination of toxicants, the solution of the differential equations[6,7] for the risk rate can be written in the form

$$r(E,\tau) = m_2[\dot{d}_{m,2}(\tau),\tau] \int_0^\tau C_1[\dot{d}_{g,1}(\xi),\xi]\, m_1[\dot{d}_{m,1}(\xi),\xi] \exp\{\int_\xi^\tau g_2[\dot{d}_{g,2}(x),x]\, dx\}\, d\xi \,. \quad (17)$$

In the framework of this model, agents that influence the birth and death rate of the sensitive normal cells, and thus increase the number, C_1, of susceptible cells, are called co-carcinogens; those that increase the transformation rate, m_1, are initiators; those that increase α_2, and thus g_2, are promoters; those that increase β_2, and thus decrease g_2, are termed inhibitors; and finally, those that increase m_2 are called completers.[6-8] A combination of exposures may, therefore, increase the total risk by different agents, enhancing the same or different transition rates, that is, by acting in various capacities in terms of the definitions above.

A relatively simple calculation for time-independent, lifelong exposures to a combination of a co-carcinogen (index 1), a predominant initiator (index 2), and a predominant completer (index 3), yields a risk rate for a tumor at age t,

$$\dot{r}_{tot}(t) = \dot{r}_0(t) \frac{C_1(\dot{d}_{g,1})\, m_1(\dot{d}_{m,2})\, m_2(\dot{d}_{m,3})}{C_1(0)\, m_1(0)\, m_2(0)} , \tag{18}$$

where $\dot{r}_0(t)$ is the incidence rate of the health effect at age t. With the approximations

$$C_1(\dot{d}_{g,k}) = C_1(0)\, [1 + f_k(\dot{d}_{g,k})] ,$$

and

$$m_i(\dot{d}_{m,k}) = m_i(0)\, [1 + f_k(\dot{d}_{m,k})] , \tag{19}$$

for the effect of toxic agents k on the susceptible normal population C_1 and the transition rates m_i, the structure of Equation 15 is obtained. The risk rate is then

$$\dot{r}_{tot}(t) = \dot{r}_0(t) \prod_{k=1}^{3} \{1 + f_k(\dot{d}_k)\} , \tag{20}$$

and the accumulated risk to age t is

$$r_{tot}(t) = r_0(t) \prod_{k=1}^{3} \{1 + f_k(\dot{d}_k)\} . \tag{21}$$

With several indirect-acting agents or mixtures of several direct- and indirect-acting agents, as in Figures 2B and 2C, the combined action of many toxicants can have the same separable structure, and the predictive power of this model is very good. Exposures to promoters and inhibitors, on the other hand, do not exhibit such a simple, separable structure. Even then, however, the prediction of the separable interaction model can be acceptable as long as the nonseparable contribution is small. Thus, there is, on the whole, considerable theoretical support for the separable interaction model, but none for the independent action model.

DISCUSSION

Two models for the combined effects of several toxic agents are presented, assuming completely independent action and separable interaction for the toxicants. The first is the obvious yardstick for determining the presence of interactions; the second provides a measure of the strength of that interaction. The models apply to quantal, "nonsignature" health effects, and provide a probability for the incidence of a disease, not for its severity. The latter would require a data base far beyond the information available at present.

As implied by the expression "nonsignature health effect", the target diseases do not differ in any way that would allow one to identify the exposure to a specific agent as the cause of a particular case of the disease. An example is an individual with leukemia and a history of exposure to both low LET radiation and benzene. Strict causality, in a legal sense, is no longer a meaningful concept, and the combined action needs to be accounted for. An expansion of this work to include a calculation of the assigned shares[5] for each of the K+1 potential causes is needed for both models.

In view of the facts that in all four cases sufficiently analyzed to date, the separable interaction model provided a good, if not the best fit,[1-3] and that the new models of carcinogenesis provide some theoretical support, a certain predictive power can be assumed for model calculations based on the marginal risks for each agent. This is done in the following paper (this report, pp. 510-515) in which the case of cancer of the esophagus as a function of exposures to alcohol, tobacco residue and radiation is discussed.

Despite the complexity of experiments involving a combination of several toxicants, it may be possible to verify some of the predictions of these models. This is possible because the verification can be used on a limited set of dose-effect data. An exposure to two toxic agents requires close to nine dose combinations for an analysis,[1] exposures to three or more agents need correspondingly more. However, with predictions available, a much lower number of measurements at critical dose combinations should allow an optimal, cost-efficient verification.

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