Interaction Between Two Carcinogens in the Two–Stage Clonal Expansion Model of Carcinogenesis

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Abstract

Exposure to two or more carcinogens may result in interactive effects in which the joint effect may, in some sense, be greater or less than the sum of the effects of the two agents alone. In this article, we investigate the joint effects of exposure to two carcinogens within the context of the two–stage clonal expansion model of carcinogenesis. Different measures of interaction are considered based on the notions of response and dose additivity, and an index of synergy S due to Thomas (1982) used to broadly characterize the effects of joint exposure. Interactive effects based on the index S were found to be qualitatively similar regardless of whether cancer risk was defined in terms of age-specific relative risk or the cumulative probability of cancer occurrence at the same age. For joint exposure to two initiators or to two completers (affecting the first or second mutation rate in the two-mutation model, respectively), S assumed values near zero, reflecting an additive relative risk relationship. For joint exposure to two promoters (which increase the rate of proliferation of initiated cells that have sustained the first mutation), the relative risk relationship was found to range from supra-multiplicative ($S > 1$) in younger age groups to sub-additive ($S < 0$) at older ages. Other combinations of carcinogens involving promotion also displayed a broad range of interaction effects. These results differ markedly from those reported previously by Kodell *et al.* (1991) for an approximate form of the two–stage model, which predicts much higher values of the index of synergy S than the exact form of the model when promotion is involved.

1 Introduction

Although many risk assessment applications focus on the effects of exposure to a single risk factor, the effects of joint exposure require investigation because of the potential for interaction between two or more agents (Krewski and Thomas, 1992). Interaction occurs when the effects of joint exposure to two agents is, in some sense, greater (synergism) or less (antagonism) than the sum of the effects of the two agents alone. Considerable experimental evidence has been accumulated to date on the joint effects of exposure to mixtures of toxic substances, including chemical carcinogens (Arcos *et al.*, 1988; Bhageri *et al.*, 1988; Rao *et al.*, 1989).

Kodell *et al.* (1991) used the Moolgavkar–Venzon–Knudson two–stage clonal expansion model of carcinogenesis to investigate the effects of exposure to two carcinogens. This model is based on the theory that a cancer cell is produced following the occurrence of two mutations in a single stem cell, with cells that have sustained the first mutation (initiated cells) subject to a birth–death process leading to clonal expansion (Moolgavkar and Luebeck, 1990). The two–stage model has been employed in

a number of applications in cancer risk assessment, and provides a useful biologically based approach to cancer risk modeling (Moolgavkar *et al*, 1999).

The work of Kodell *et al.* (1991) was a follow–up to a previous study of interaction conducted by Brown and Chu (1989) using the Armitage–Doll multistage model of carcinogenesis. In both studies, the effects of joint exposure were evaluated with respect to additive and multiplicative models of relative risk, with risk defined in terms of the widely used approximate form of the age-specific hazard functions for the two–stage and multi–stage models. Although a number of interesting characterizations of the effects of joint exposure were discussed in the above studies, Kodell *et al.* (1991) pointed out that the implications for health risk assessment needed further evaluation, since risk assessment applications generally focus on the cumulative (lifetime) probability of an adverse health effect, rather than on age-specific hazard rates.

The present paper explores joint carcinogenic action within the context of the two–stage clonal expansion model in terms of both the exact age–specific hazard function and the exact cumulative hazard. The traditional toxicological concepts of dose additivity and response additivity are shown to share common ground with the additive and multiplicative models of relative risk as baseline models of non-interactive joint action. An index of synergy due to Thomas (l982) is shown to be particularly useful in characterizing the effects of joint exposure to two carcinogens.

2 Exact Form of the Two–Stage Model

In this section, we present expressions for exact and approximate forms of the two–stage model of carcinogenesis which are needed to evaluate interactive effects. Following Moolgavkar *et al.* (1988), we let $X(t), Y(t)$, and $Z(t)$ represent the number of susceptible stem cells, intermediate cells, and malignant cells, respectively, in the target tissue at time t. The first and second stage transition rates will be denoted by $\nu(t)$ and $\mu(t)$, respectively, with $\alpha(t)$ and $\beta(t)$ denoting the birth and death rates of intermediate cells. Let us denote probability of having exactly j intermediate cells and k malignant cells at time t by $P_{j,k}(t,t_0) = Prob\ [Y(t) = j, Z(t) = k | Y(t_0) = 0, Z(t_0) = 0]$, given there are no intermediate and no malignant cells at time t_0.

The probability generating function

$$\Psi(y,z;t,t_0) = \sum_{j,k}\ P_{j,k}(t,t_0) y^j z^k \tag{1}$$

satisfies the Kolmogorov forward equation

$$\begin{aligned}\Psi_t(t,t_0) &= (y-1)\nu(t)X(t)\Psi(t,t_0) \\ &+ [\mu(t)yz+\alpha(t)y^2+\beta(t)-\{\alpha(t)+\beta(t)+\mu(t)\}y]\Psi_y(t,t_0)\end{aligned}$$

(Moolgavkar *et al*, 1988), with initial condition $\Psi(y,z,t_0,t_0)=1$ The probability of at least one malignant cancer cell appearing by time t is $P(t)=1-\Psi(1,0;t)$. The hazard function, which represents the instantaneous rate of appearance of a malignant cell in previously cancer free tissue, is given by

$$h(t) = -\Psi'(1,0;t)/\Psi(1,0;t), \tag{2}$$

where derivative is taken with respect to time t. It follows from the Kolmogorov equation that

$$\Psi'(1,0;t) = -\mu(t)\frac{\partial\Psi}{\partial y}(1,0;t) \tag{3}$$

with

$$E[Y(t) \mid Z(t)=0] = \frac{\partial\Psi}{\partial}(1,0;t)/\Psi(1,0;t), \tag{4}$$

where E denotes expectation. Thus, the hazard function can be expressed as

$$h(t) = \mu(t)E[Y(t) \mid Z(t)=0]. \tag{5}$$

When the probability of tumor occurrence is small, $E[Y(t) \mid Z(t)=0] \approx E[Y(t)]$, and $h(t)$ may be approximated by

$$h(t) \approx \mu(t)\int_0^t \nu(v)X(v)\exp\Big\{\int_v^t(\alpha(u)-\beta(u))du\Big\}dv. \tag{6}$$

This approximate form of hazard function has been widely used in applications of the two–stage model (Portier and Bailer; 1989, Kodell *et al.*, 1991) because of its simplicity.

The exact hazard function can be computed using the probability generating function Ψ. The survival function, $S(t)=1-P(t)$, can be written in the form

$$S(t) = \Psi(1,0;t) = \exp\int_0^t [y(u,t)-1]\nu(u)X(u)du, \tag{7}$$

where the dependence of y on u and t is explicitly acknowledged. The hazard function then is given by

$$h(t) = -\Psi'(1,0;t)/\Psi(1,0;t) = -\int_0^t \nu(u)X(u)y_t(u,t)du, \tag{8}$$

where y_t denotes the derivative of y with respect to t.

If the transition rates in the model are functions of time t, it is not possible to give an explicit expression for $y(u,t)$, and hence for Ψ and $h(t)$. In general, y satisfies a Riccati differential equation with variable coefficients for which only numerical solutions are available (Quinn, 1989).

However, when the rates are piecewise constant, the Riccati equation can be integrated to yield a closed form expression for $y(u,t)$ (Moolgavkar *et al.*, 1993). The equations for $\Psi(1,0;t)$ and $h(t)$ can be numerically integrated using the values of $y(u,t)$ and $y_t(u,t)$. When $\nu(u)$ is piecewise constant and $X(u)$ is constant, then the equation for $\Psi(1,0;t)$ can be solved in closed form, and the hazard $h(t)$ can be easily computed numerically as a finite difference.

Unlike the exact form of the hazard function for the two–stage model, the approximate hazard depends only on the difference $\delta(t) = \alpha(t) - \beta(t)$ between the birth and death rates of intermediate cells. This net birth rate effectively represents the rate of promotion of intermediate cells. Moolgavkar *et al.* (1988) point out that the approximate hazard can overestimate the exact hazard substantially. The approximate and exact hazards exhibit qualitatively different behavior: whereas the approximate hazard increases without bound with time t, the exact hazard reaches a maximum value and subsequently declines.

Thorslund *et al.* (1987) used the approximate form of the two–stage model to classify carcinogens as initiators, promoters or completers. Specifically, an initiator is an agent that increases the rate of occurrence ν of the first genetic event, while a completer increases the rate of occurrence μ of the second genetic event, i.e., cellular transformation to a malignant state. A promoter increases the difference $\delta = \alpha - \beta$ between the birth and death rates of initiated cells, leading to clonal expansion. In contrast to initiators and completers which act by genotoxic means, promoters may exert their effects through non–genotoxic mechanisms such as the stimulation of cellular proliferation as a consequence of cytotoxicity.

3 Interaction Between Two Carcinogens

3.1 Dose Additivity and Response Additivity

The concept of additivity underlies almost all investigations of the joint toxic action of chemicals (Kodell and Pounds, 1991) or drug combinations (Unkelbach and Wolf, 1985). Identical biological action of two chemicals (i.e., when one chemical is simply a dilution of the other) implies dose additivity, whereas biological and statistical independence of action implies response additivity.

Let $P(t; d_1, d_2)$ denote the probability of cancer from exposure to a dose d_1 of chemical C_1 and a dose d_2 of chemical C_2 at time t. Under dose additivity, $P(t; d_1, d_2) = P(t; d_1 + \rho d_2, 0) = P(t; 0, \rho^{-1} d_1 + d_2)$, where $\rho = d_1/d_2$ is the potency of C_2 relative to chemical C_1. Under response additivity,

$$\begin{aligned} P(t; d_1, d_2) &= P(t; d_1, 0) + [1 - P(t; d_1, 0)]P(t; d_2, 0) \\ &= P(t; 0, d_2) + [1 - P(t; 0, d_2)]P(t; d_1, 0) \\ &= P(t; d_1, 0) + P(t; 0, d_2) - P(t; d_1, 0)P(t; 0, d_2). \end{aligned}$$

Note that $P(t; d_1, d_2) \approx P(t; d_1, 0) + P(t; 0, d_2)$ under response additivity when either $P(t; d_1, 0)$ or $P(t; 0, d_2)$ is small.

Assume that the rates of occurrence of the two genetic events (mutations) and the rate of clonal expansion in the approximate form of two–stage clonal expansion model are linearly related to dose. Thus, for an initiating agent, $\nu(t) = a_0 + a_1 d_i(t)$, where $d_i(t)$ is the dose of the initiator at time t. Similarly, $\mu(t) = b_0 + b_1 d_c(t)$ for a completer, and $\delta(t) = c_0 + c_1 d_p(t)$ for a promoter. For two initiators acting together $\nu(t) = a_0 + a_{1i} d_i(t) + a_{1i'} d_{i'}(t)$, where $d_i(t)$ and $d_{i'}(t)$ indicate the dose of initiator i and i' at time t, respectively. Similar definitions apply for two promoters or two completers.

Observe that under the present formulation, the joint action of two initiators would be dose additive, as would the joint action of two completers or of two promoters. The potency ρ of C_1 relative to C_2 would be defined by $\rho = a_{1i'}/a_{1i}$ for two initiators, with similar definitions for two completers and two promoters. Hence, the concept of dose additivity has a natural interpretation within the context of the two–stage clonal expansion model as formulated here. The additivity of two initiators or two completers also holds in the exact form of the two–stage model, since these results depend only on linear dependence of the two mutation rates on the dose of the two

agents of interest. The case of two promoters is more involved under the exact form of the two–stage model, since dose dependencies of the birth rate $\alpha(t)$ and death rate $\beta(t)$ of intermediate cells both need to be specified.

Interactive effects are commonly examined in epidemiological studies of risk factors for cancer in human populations. A widely used epidemiological measure relating exposure to a specific risk factor and the disease under study is the relative risk (RR), defined as the risk in the presence of exposure relative to the risk in the absence of exposure. Let $RR(t; d_1, d_2)$ denote the relative risk of cancer from exposure to a dose d_1 of chemical C_1 and a dose d_2 of chemical C_2 at the age t. In particular, let $RR(t; 0, d_2)$ denote the relative risk of cancer from exposure to a dose d_2 of chemical C_2 at the age t. Similarly, let $RR(t; d_1, 0)$ denote the relative risk of cancer from exposure to a dose d_1 of chemical C_1 at age t.

The relative risk can than be defined in terms of the age specific hazard

$$RR(t; d_1, d_2) = h(t; d_1, d_2)/h(t; 0, 0) \tag{9}$$

or the probability of cancer by age t

$$RR(t; d_1, d_2) = P(t; d_1, d_2)/P(t; 0, 0). \tag{10}$$

Two special cases of particular interest are the additive relative risk model

$$RR_+(t; d_1, d_2) = RR(t; d_1, 0) + RR(t; 0, d_2) - 1 \tag{11}$$

and multiplicative relative risk model

$$RR_\times(t; d_1, d_2) = RR(t; d_1, 0) \times RR(t; 0, d_2). \tag{12}$$

The additive relative risk model defined in terms of age–specific hazard is related to response additivity. Specifically, it can be shown that the additive relative risk model based on age–specific hazards implies that

$$P(t; d_1, d_2) = \frac{P(t; d_1, 0) + P(t; 0, d_2) - P(t; d_1, 0)P(t; 0, d_2) - P(t; 0, 0)}{1 - P(t; 0, 0)}, \tag{13}$$

where $P(t; 0, 0)$ is the probability of cancer in the absence of exposure to either chemical. Thus, the additive relative risk model based on age–specific hazards approximates response additivity when the background risk $P(t; 0, 0)$ is small.

Further motivation for the concept of response additivity is obtained by considering continuous joint lifetime exposure to an initiator and a completer at constant rates d_i and d_c respectively. For the multiplicative relative risk relationship defined in terms of the age-specific hazards, it can be shown that if $a_0 b_0 = a_1 d_i b_1 d_c$, then response additivity holds exactly under the approximate form of the two–stage model. Notice that one way for this condition to hold is for $a_0 = a_1 d_i$ and $b_0 = b_1 d_c$, which corresponds to a doubling of each spontaneous mutation rate as a consequence of exposure to dose d_i of the initiator and dose d_c of the completer. Although dose additivity and response additivity may coincide, at least approximately, in certain cases (e.g., additive relative risk for two initiators with low background risk), they represent different approaches to characterizing interaction (Kodell and Pounds, 1991). Since both concepts are justifiable as baseline models of non-interactive joint effects, it might seem desirable to interpret joint carcinogenic action with respect to departures from both of these benchmarks of additivity.

For present purposes, response additivity provides a convenient primary benchmark against which to evaluate joint carcinogenic action under the two–stage clonal expansion model. This is because response additivity provides a general approach to risk assessment for joint effects that can be formulated for any combination of carcinogens, whether or not the mechanisms of action of individual carcinogens is known or postulated. With respect to dose additivity, however, there are cases for which knowledge of mechanisms of action of individual carcinogens precludes the formulation of a dose-additive joint carcinogenic response (e.g., initiator plus completer). Nonetheless, dose additivity is recognized as an important alternative concept of additivity that can be exploited when common mechanisms of action are known or expected.

3.2 Thomas' Index of Synergy

Note that while $RR(t; d_1, d_2)$ is estimated from observed data, $RR_+(t; d_1, d_2)$ and $RR_\times(t; d_1, d_2)$ are predicted from the relative risks for each of the two agents to which exposure may occur. Thus, after determining $RR(t; d_1, 0)$, $RR(t; 0, d_2)$ and $RR(t; d_1, d_2)$ from empirical data, we can calculate $RR_+(t; d_1, d_2)$ and $RR_\times(t; d_1, d_2)$. By comparing the observed value of $RR(t; d_1, d_2)$ to $RR_+(t; d_1, d_2)$ and $RR_\times(t; d_1, d_2)$, we can infer if an effect is more likely to be additive, multiplicative, or otherwise.

To facilitate such analyses, Thomas (1982) proposed the index of synergy

$$S = \frac{RR(t; d_1, d_2) - RR_+(t; d_1, d_2)}{RR_\times(t; d_1, d_2) - RR_+(t; d_1, d_2)} \quad . \tag{14}$$

$S = 0$ corresponds to the baseline case of additive relative risk. Values of $S < 0$ reflect sub–additivity or antagonism. Supra–additivity ($S > 0$) corresponds to synergistic interaction: values near $S = 1$ suggest multiplicative risks and agents which combine with supra–multiplicative effects are characterized by values of $S > 1$.

4 Interactive Effects under the Two–Stage Model

4.1 Theoretical Results for the Approximate Solution

Kodell *et al.* (1991) investigated interactive effects under the approximate form of the two–stage model. In particular, they studied relative risk relationships for joint exposures to two carcinogens (initiators, promoters, or completers) under two general exposure scenarios. The first was simultaneous lifetime exposure to both agents. This type of exposure is considered to be an appropriate baseline for studying joint exposure relationships, and is encountered in many toxicologic and epidemiological studies. The second was non–overlapping partial lifetime exposures.

Age–specific relative risk relationships for joint exposures to two carcinogens were explored for each of these exposure scenarios, with relative risk defined in terms of age-specific hazards. Specifically, for an initiating carcinogen, $\nu(t) = a_0 + a_1 d_i(t)$, where $d_i(t)$ is the dose of the initiator at time t. Note that since a_0 does not depend on time t, the spontaneous initiation rate $\nu \equiv a_0$ is assumed to be constant over time. For a completer, $\mu(t) = b_0 + b_1 d_c(t)$, where $d_c(t)$ is the dose level of the completer at time t. For two initiators acting together, $\nu(t) = a_0 + a_{1i} d_i(t) + a_{1i'} d_{i'}(t)$, with a similar expression holding for the second mutation rate μ with joint exposure to two completers. Upon exposure to a promoter, it is assumed that the rate of clonal expansion is given by $\delta_0(t) + f_p(d_p(t))$, where $f_p(d_p(t))$ is an increasing function of dose $d_p(t)$ of the promoter and $\delta_0(t)$ is the background rate of proliferation of initiated cells at the time t. The functional form of f_p and $\delta_0(t)$ may remain unspecified.

The results of Kodell *et al.*(1991) are summarized in Table 1. For two initiators or two completers, the age–specific relative risk is additive, regardless of the exposure scenario considered. However, the age–specific relative risk for an initiator and completer is multiplicative. When one or both of the two agents is a promoter, supra–multiplicative interactions can occur.

Table 1. Interactive Effects for Joint Exposure to Two Carcinogens Under the Approximate Form of the Two–Stage Model.

Carcinogen C_1	Carcinogen C_2	Interaction
Initiator	Initiator	Additive
Completer	Completer	Additive
Initiator	Completer	Multiplicative
Promoter	Promoter	Supra–multiplicative
Initiator	Promoter	Multiplicative to supra–multiplicative

4.2 Empirical Results for the Exact Solution

Moolgavkar *et al.* (1993) used the exact form of the two–stage model to describe the interaction between radon and tobacco smoke in the induction of lung cancer in underground miners. Radon, the second leading cause of lung cancer in the general population after tobacco smoke, exhibits a high degree of synergism with tobacco smoke (National Research Council, 1999), as will be demonstrated here. The transition intensity functions rates corresponding to the first and the second mutation rates were modeled as linear functions

$$\nu(d_s, d_r) = a_0 + a_s d_s + a_r d_r \tag{15}$$

and

$$\mu(d_s, d_r) = b_0 + b_s d_s + b_r d_r, \tag{16}$$

where d_s and d_r represent the level of exposure to tobacco smoke and radon respectively. (Both d_r and d_s may vary with age.) The rate of promotion was modeled as the nonlinear function

$$\begin{aligned}(\alpha - \beta)(d_s, d_r) &= c_0 + c_{s1}(1 - \exp[-c_{s2} d_s]) \\ &+ c_{r1}(1 - \exp[-c_{r2} d_r]),\end{aligned}$$

with β/α held constant. This functional form allows for saturation of the effects of both radon and tobacco smoke. Since no effect of radon or smoking on the second mutation rate was observed, b_s and b_r were set to zero. With the identifiability constraint $a_0 = b_0$, only nine parameters were actually estimated. The maximum likelihood estimates of these parameters are given in Table 2 (Moolgavkar *et al.*, 1993).

Table 2. Estimates of the Paramaters of the two–stage Model fit to the Colorado Uranium Miners Data

Parameter	Estimate
$a_0 = b_0$	1.11×10^{-7}
a_s	1.44×10^{-8}
a_r	2.51×10^{-8}
c_0	1.10×10^{-1}
c_{s1}	4.93×10^{-2}
c_{s2}	1.67×10^{-1}
c_{r1}	4.16×10^{-1}
c_{r2}	7.09×10^{-2}
β/α	9.93×10^{-1}

The relative risks of lung cancer at age 60 based on the fitted two–stage model are given in Table 3 for both single and joint exposures to radon and cigarette smoke, along with Thomas' index of synergy. For both the age–specific hazard–based and cumulative probability–based definitions of relative risk, the index of synergy indicates that the relative risks are supra-additive but sub-multiplicative. Further, the pattern of relative risks for these two measures of risk are qualitatively similar, showing close agreement in the characterization of the joint effects of radon and cigarette smoke. These results suggest that similar interpretations of joint carcinogenic action will arise, regardless of whether relative risk is defined in the epidemiological tradition using age-specific relative risk or in the toxicological tradition using cumulative probability.

Further insight into radon-tobacco interactions can be gained by examining the plots of the indices of synergy over time shown in Figure 1 for the exposure scenarios considered in Table 3. Note that the indices of synergy S in both Figure 1a (based on the exact age–specific hazard) and Figure 1b (based on exact cumulative probability of cancer) tend to cluster together according to the level of exposure to radon (high or low). The level of exposure to cigarette smoke does not appear to have an appreciable effect on S, after taking into account the level of exposure to radon. For high radon exposure, there is a marked decline in the S around age 40 where exposure to radon stops. Supra–additivity is much more pronounced at younger ages after exposure commences than at older ages, with the relative risks being essentially additive at extreme ages. Except for the earlier ages in Figure 1b, the values of S reflect a sub–multiplicative interaction between radon and tobacco. Based on this example,

it appears that at the more extreme ages where joint effects are normally evaluated, relative risks based on age–specific hazards will lead to similar interpretations, at least qualitatively, to those based on cumulative probabilities of tumor occurrence.

Table 3.1 Relative Risk of Lung Cancer due to Exposure to Radon and Tobacco Smoke Based on Exact Age–Specific Hazard[a]

Radon Exposure[b] (WLM/month)	Tobacco Exposure[c] (cigarettes/day)	Relative Risk due to Radon	Relative Risk due to Tobacco	Relative Risk for Combined Exposure	Index of Synergy S
1.0	10	1.3	5.4	6.6	0.68
1.0	30	1.3	10.2	12.3	0.70
1.0	40	1.3	11.8	14.4	0.73
50.0	10	12.9	5.4	28.0	0.21
50.0	30	12.9	10.2	46.8	0.23
50.0	40	12.9	11.8	55.3	0.25

a. Relative risk evaluated at 60 years of age
b. Exposure to radon between 30 and 40 years of age
c. Cigarette smoking between 15 and 60 years of age

Table 3.2 Relative Risk of Lung Cancer due to Exposure to Radon and Tobacco Smoke Based on Exact Cumulative Probability[a]

Radon Exposure[b] (WLM/month)	Tobacco Exposure[c] (cigarettes/day)	Relative Risk due to Radon	Relative Risk due to Tobacco	Relative Risk for Combined Exposure	Index of Synergy S
1.0	10	1.3	4.4	5.5	0.84
1.0	30	1.3	7.8	9.7	0.83
1.0	40	1.3	8.9	11.1	0.84
50.0	10	20.4	4.4	42.3	0.28
50.0	30	20.4	7.8	65.0	0.29
50.0	40	20.4	8.9	73.4	0.30

a. Relative risk evaluated at 60 years of age
b. Exposure to radon between 30 and 40 years of age
c. Cigarette smoking between 15 and 60 years of age

4.3 Further Numerical Results for the Exact Solution

In this section, we extend the results of Kodell *et al.* (1991) for the approximate form of the two–stage model to the exact form. For convenience, we will use the parameter values obtained by fitting the exact form of model to the Colorado miners

data discussed in the previous section as a starting point in for further numerical study. Since the parameters b_s for the second mutation rate were equal to zero, we set $b_s = a_s$ and $b_r = a_r$ to enrich our numerical investigation. Although this parameter specification no longer pertains directly to radon and lung cancer, it does provide a set of plausible parameter values on which we base our numerical exploration of interactive effects, including those involving completers that increase the second stage mutation rate.

We investigate interactions between two carcinogens using the index of synergy S defined in terms of either age-specific hazard or cumulative probability of cancer by age t. Patterns of interaction between two carcinogens acting on a single component (initiation, promotion or completion) were investigated under a simple exposure scenario, with exposure to either carcinogen starting at age 15 years of age and continuing at the same level through to 80 years of age. Only the relevant parameters from Table 2 are used in each case: with two initiators, for example, we use $a_0 = b_0$, a_s and a_r as in Table 2, with all remaining parameters set to zero.

The index of synergy S based on the age-specific hazard is shown in Figure 2 for four different carcinogen combinations. The interaction between two initiators conforms closely to additivity for all t. The case of constant lifetime exposure to two completers (not shown in Figure 2) follows essentially the same pattern as that for two initiators. Unlike the cases of exposure to two initiators or two completers, in which the risks are close to additive, a near multiplicative relative risk relationship arises in the initiator–completer paradigm. For large t, however, the relationship becomes supra–multiplicative.

The negative values of S that occur at older ages in the case of two promoters warrant comment. This reflects a situation in which the joint effect of the two promoters has resulted in the formation of a large number of intermediate cells at earlier ages. The apparent antagonism ($S < 0$) at older ages between the two promoters is thus mediated by the high probability that the first malignant cell arises from this large pool of intermediate cells at an early age.

The difference between the behavior of the index of synergy for the approximate form of the two–stage model (not shown) and the exact form of the model is dramatic. Under the approximate model, the relative risk for continuous lifetime exposure to two promoters is always supra–multiplicative. The exact version of the model implies

values of S slightly above 1 (supra–multiplicativity), followed by decline below 0 (sub–additivity) for larger t. The approximate model predicts that the relative risk for simultaneous exposure to an initiator and a promoter is multiplicative to supra–multiplicative, whereas under the exact model, the index S varies over a wide range, including sub-multiplicative and sub-additive effects.

The index of synergy S based on cumulative tumor probability is shown in Figure 3. The behaviors of S based on cumulative probability generally mirrors that based on age–specific hazard. The interaction between two initiators is additive. The same pattern is observed with constant lifetime exposure to two completers (not shown).

All other combinations in Figure 3 exhibit a sharp jump in the index S to values above 1 immediately following the commencement of exposure at age 15. In the case of exposure to an initiator and a completer, S quickly jumps to a value of over 6, declines to its lowest level of 1.1 at age 44, and then increases slowly until age 80. For the initiator–promoter paradigm, S declines from a value of nearly 4 at age 15 to its lowest value of 0.5 at age 30. This is followed by a slow increase to $S > 1$ and another decline to 1.2 at age 80. The index of synergy for two promoters declines steadily from a level above 1 (supra–multiplicativity) to slightly below 0 (sub–additivity) for larger t. However, the apparent antagonism between two promotors when the index S is based on cumulative risk (Figure 3) is less pronounced than when using age–specific risk (Figure 2).

Although the results presented in Figure 2 and Figure 3 imply qualitatively similar interpretations, there are clear quantitative differences of the index of synergy defined in terms of either age–specific hazard or cumulative probability of tumor occurrence.

5 Discussion

The assessment of risk due to joint exposure to two or more carcinogens is of concern because of the potential for synergism between the agents involved, as occurs with the induction of lung cancer due to joint exposure to radon and tobacco smoke (National Research Council, 1999). For risk assessment purposes, it is important to identify whether the effects of joint exposure are synergistic, additive, or even antagonistic. Although a single universal definition of interaction is not possible because of the complexity of interactive effects, the notions of dose additivity and response additivity provide useful baselines against which to assess synergistic or antagonistic departures

from additivity.

In order to characterize interaction, it is necessary to specify the scale on which risk is measured. In many epidemiological applications, age-specific relative risk is used. In contrast, toxicological risk assessment has focused more on the cumulative probability of tumor occurrence by a specified age (often corresponding to the expected lifespan of the test species). Because both scales have merit, we have included both in our investigation of interaction. The index of synergy S due to Thomas (1982), which reflects departures from additive or multiplicative relative risk relationships, can be used regardless of whether relative risk is defined in terms of age-specific hazard or cumulative tumor probability.

Brown and Chu (1989) considered the effect on S of combinations of carcinogens using the approximate form of the Armitage–Doll multistage model. Only two carcinogens were considered, with the two agents of interest affecting the same or different stages. Exposures that affect the same stage produce additive (age–specific) relative risks. Limited duration exposures which affect different stages tend to yield a supra–additive but sub–multiplicative interaction. Thus, the observation of a multiplicative interaction suggests that the two agents affect different stages. However, additivity can occur with two agents affecting either different stages or the same stage.

The focus of the present paper has been the evaluation of interaction within the context of the two–stage clonal expansion model of carcinogenesis. Whereas Kodell *et al.* (1991) previously addressed the issue of interaction between two carcinogens using the approximate solution to the two–stage model, the present paper is based on the exact solution. The choice of model parameters was guided by a previous application of the exact form of the two–stage model in describing the joint effects of exposure radon and tobacco smoke based on epidemiologic data on uranium miners in the Colorado Plateau.

In general, the temporal patterns of interaction were qualitatively similar regardless of whether the relative risk was based on age-specific hazard or cumulative probability. For joint exposure to two initiators or to two completers, the values of the index of synergy S were calculated to be near zero, reflecting an additive relative risk relationship. For joint exposure to two promoters, the relative risk relationship was found to range from supra-multiplicative ($S > 1$) in younger age groups to sub-additive ($S < 0$) at older ages. Other combinations of carcinogens involving

promotion displayed a similarly broad range of interaction effects.

While the index of synergy S used here is based on traditional epidemiologic (age-specific risk) and toxicologic (cumulative risk) indicators of risk, the values of the index reported here were calculated within the context of the two–stage clonal expansion model of carcinogenesis. In general, the values of the index S reflecting additivity, synergism or antagonism appear compatible with intuitive notions of how two carcinogens might interact within the context of this biologically based model of carcinogenesis: additivity with two initiators, multiplicativity with an initiator and completer, and supra-additivity to supra-multiplicativity with an initiator and promoter. The one exception occurs with two promoters, for which the index S indicates a synergistic effect at early ages and an apparent antagonistic effect at older ages due to the large pool of intermediate cells available for malignant conversion at early ages. This result is not necessarily a limitation of the index S, but rather an indication of the insight afforded by the use of a biologically based model of carcinogenesis in explaining an otherwise anomalous result based on a conventional index of synergy computed on the basis of age-specific or cumulative risk.

These results differ markedly from those reported previously by Kodell *et al.* (1991) for the approximate form of the two–stage model, which predicts much higher values of the index of synergy S than the exact form of the model when promotion is involved. The fact that maximal discrepancies between the approximate and exact forms of the two–stage model occur when one or both agents is a promoter is not surprising, since the approximation used involves the rate of clonal expansion of initiated cells.

Biologically based cancer risk models continue to evolve as our understanding of the process of carcinogenesis increases (Moolgavkar *et al.*, 1999). Denes and Krewski (1995) have extended the two–stage clonal expansion model to allow for stochastic rather than deterministic stem cell growth. Although some form of dampened stochastic model for stem cell growth may be more realistic, this work provides the mathematical basis for a three–mutation model with deterministic stem cell growth. Zheng *et al.* (1997) has developed the requisite mathematics to permit the analysis of multi–stage models with clonal expansion at several intermediate stages. Dewanji *et al.* (1999) recently developed methods for incorporation information on the number and size of premalignant clones in fitting the two–stage model in longitudinal studies.

As practical experience with more elaborate biologically based models of carcinogenesis accumulates, the issue of interaction should be revisited. Further investigations along these lines would serve to establish the generalizability of our results. In addition to examining other biologically based models of carcinogenesis and additional exposure scenarios that might be of interest, the effect of competing risks on the patterns of interaction (Dewanji *et al.*, 1993; Kodell *et al.*, 1986) should also be examined. We hope this initial investigation focusing on the two–stage clonal expansion model of carcinogenesis will stimulate further work in this area, ultimately leading to a better understanding of the effects of joint exposure to two or more carcinogens, and better methodologies for assessing the risk of joint exposures.

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7 References

Arcos, J.C., Woo, Y.T., and Lai, D.Y.(1988). Database of binary combination effects of chemical carcinogens. Environmental Carcinogenesis Reviews C6: 1–148.

Bagheri, D., Doeltz, M.K., Fay, J.R., Helmes, C.T, Monasmith, L.A, and Sigman, C.C. (1988). Database of inhibitors of carcinogenesis. Environmental Carcinogenesis Reviews C6: 261–378.

Brown, C.C. & Chu, K.C. (1989). Additive and multiplicative models and multistage carcinogenesis theory. Risk Analysis 9: 99-105.

Denes, J. & Krewski, D. (1995). An exact representation of the generating function for the Moolgavkar-Venzon-Knudson two–stage model of carcinogenesis with stochastic stem cell growth. Mathematical Biosciences 131: 185-204.

Dewanji, A. & Krewski, D. & Goddard, M.J. (1993). A Weilbull model for for the estimation of tumorigenic potency. Biometrics 49: 367-377.

Dewanji, A., Goddard, M.J., Krewski, D. & Moolgavkar S.H. (1999). Two stage model for carcinogenesis: number and size distributions of premalignant clones in longitudinal studies. Mathematical Biosciences 155: 1-12.

Kodell, R.L, Gaylor D.W. & Chen J.J. (1986). Standardized tumor rates for chronic bioassays. Biometrics 42: 867-873.

Kodell, R.L, Krewski D. & Zielinski J.M. (1991). Additive and multiplicative relative risk in the two–stage clonal expansion model of carcinogenesis. Risk Analysis 11: 483-490

Kodell R.L. & Pounds J.G. (1991). Assessing the toxicity of mixtures of chemicals. In: D. Krewski and C.A. Franklin, eds. Statistical Methods in Toxicological Research. Gordon and Breach, New York, pp. 557-589.

Krewski, D., Thorslund, T. & Withey, J. (1989). Carcinogenic risk Assessment of complex mixtures. Toxicology and Industrial Health 5: 851-867.

Krewski, D., Goddard, M.J. & Zielinski, J.M. (1992). Dose-response relationship in carcinogenesis. In: H. Vainio, P.N. Magee, D.B. McGregor and A.J. McMichael,

eds. Mechanisms of Carcinogenesis in Risk Identification, Lyon, International Agency for Research on Cancer, pp. 579-600.

Krewski, D. & Murdoch, D.J. (1990). Cancer modeling with intermittent exposures. In: *Scientific Issues in Quantitative Cancer Risk Assessment* (S.H. Moolgavkar, ed.). Birkhauser, Boston, pp. 196-214.

Krewski, D. & Thomas, R.D. (l992). Carcinogenic mixtures. Risk Analysis 12: 105-ll3.

Moolgavkar S.H., Dewanji A. & Venzon D.J. (1988). A stochastic two–stage model for cancer risk assessment. I. Hazard function and probability of tumor. Risk Analysis 8: 383-392

Moolgavkar S.H.& Luebeck G. (1990). Two-event model for carcinogenesis: Biological, mathematical and statistical considerations. Risk Analysis 10: 323-341.

Moolgavkar S.H., Luebeck G., Krewski D. & Zielinski J.(1993). Radon, cigarette smoke and lung cancer: A reanalysis of the Colorado Plateau uranium miners data. Epidemiology 4: 204-217.

Moolgavkar, S.H., Krewski, D., Zeiss, L., Cardis, E. & Moller, H. (Eds.) (1999). The Scientific Basis for the Quantitative Estimation and Prediction of Cancer Risks to Humans. IARC Scientific Publication No. 131, International Agency for Research on Cancer, Lyon.

National Research Council. (1988). Complex Mixtures: Methods for In Vivo Toxicity Testing. National Academy Press, Washington, DC.

National Research Council (l999). Health Effects of Exposure to Radon. National Academy Press, Washington, D.C.

Portier, C. J. & Bailer, A. J. (1989). two–stage models of tumor incidence for historical control animals in the National Toxicology Program's carcinogenicity experiments. Journal of Toxicology and Environmental Health 27: 21–45.

Rao, V.R., Woo, Y.T., Lay, D.Y., & Arcos, J.C. (1989). Database on promoters of chemical carcinogenesis. Environmental Carcinogenesis Reviews C7: 145–387.

Quinn D.W. (1989). Calculating the hazard function and probability of tumor for cancer risk assessment when the parameters Are time dependent. Risk Analysis 9: 407-413.

Thomas, D.C. (1982). Temporal effects and interactions in cancer: implication of carcinogenic models. In: R. Prentice and A.S. Whittemore, eds. Society of Industrial and Applied Mathematics, Philadelphia, pp. 107-121.

Thorslund, T.W., Brown, C.C. & Charnley, G. (1987). The use of biologically motivated mathematical models to predict the actual cancer risk associated with environmental exposure to a carcinogen. Risk Analysis 7: 109-119.

Unkelbach, H.D., & Wolf, T. (1985). Qualitative Dosis-Wirkungs-Analysen. Einzelsubstanzen und Kombinationen. Fischer Verlag, Stuttgart.

Zielinski, J.M. & Krewski, D. (1992). Application of the two–stage model clonal expansion model in characterizing the joint effect of exposure to two carcinogens: In: F.T. Cross, ed. Indoor Radon and Lung Cancer: Reality or Myth? 29th Hanford Symposium on Health and Environment held October 15-19, 1990, Battelle Press, Columbus, Ohio, pp. 845-860.

Zheng, Q., Lutz, W.K. & Gaylor, D.W. (1997). A carcinogenesis model describing mutational events at the DNA adduct level. Mathematical Biosciences 144: 23-44.

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