A Semi-Parametric Model for Assessing Effects of Carcinogens on Cancer Development: Some Simulation Results

K. P. Singh, A. S. Alghamdi, A. A. Bartolucci and T. M. Smoot
Department of Biostatistics, School of Public Health
The University of Alabama at Birmingham
Birmingham, Alabama 35294-0008 U.S.A.

Abstract Several statistical methods have been proposed by scientists to describe the time to appearance and growth rate of tumors caused by environmental agents; particularly evaluation of the hazard of chemical carcinogens and its relation to the cause and resulting growth rate of tumors. The discovery of DNA opens the opportunity for scientists to understand the basic feature of the development of carcinogenicity. Consequently significant advances in modeling of effects of carcinogenesis have been achieved. The two stage models proposed by Moolgavkar and Knudson (1981) and Moolgavkar and Venzon (1979) have made significant contributions to cancer modeling because of their biological properties. In this paper, by incorporating the two-stage models of Moolgavkar-Venzon-Knudson (MVK) we propose a semi-parametric method and study its statistical and biological properties. Using simulations we study effects of carcinogens on cancer development through some initiation-promotion experiments. We also discuss the model for analysis of case-control studies for survival/sacrifice experiments.

1. Introduction

Chemicals are considered to be a major etiologic factor in the genesis of human tumors. In contrast to the benign tumor, malignant, or cancerous tumors are classified as potentially fatal. These tumors can be caused by certain agents call carcinogens. Included in the list of possible carcinogens are viruses, genetic factors, life-style, and certain chemicals (Whittemore and Keller, 1978). The extermination of possible carcinogens from our climate has become a major societal and governmental aim. Information on the carcinogenic potential of chemicals has been obtained from bioassay studies managed with animal models to understand the mechanism of carcinogenesis.

Carcinogenesis is a complex process involving two stages (Whittemore and Keller, 1978): transformation and growth. Transformation is the stages in which a normal cell has the occurrence of one or more changes or mutations which make it capable to develop cancer. Whereas growth is the process of multiplication by cell division, in which the transformed cell produces a colony of descendants called a clone. When the number of cells in the clone is large enough the clone becomes a detectable tumor. Tumors are capable of extensive growth. Carcinogenicity is possible because the cell division rate of the transformed cell is relatively higher than the death of the differentiated cell. This may result in an increase in the number of susceptible target cells. Consequently, increasing the probability that one of these target cells sustains the mutation requires for tumor transformation (Moolgavkar and Luebeck, 1992). Data from several areas of research including oncology, toxicology, and epidemiology support this multistage nature of carcinogenicity.

Since the 1950's a number of different quantitative theories of carcinogenesis have been proposed. The main objective of these theories is to relate the frequency and time of occurrence of detectable tumors to the concentration and potency of the carcinogen (Whittemore and Keller, 1978). A discussion of some of these theories can be found in Armitage and Doll (1961). In this paper, by incorporating the two mutation mode of carcinogenesis proposed by Moolgavkar and Venzon (1979) and Moolgavkar and Knudson (1981), we propose a semi-parametric model and study its properties. Using this model we study the effects of carcinogens on cancer tumor development through simulated initiation-promotion experiments.

In our analysis of both human epidemiological and experimental animal data our mathematical models of interest are the hazard (incidence) function and the probability function associated with tumor development by a given time, t.

$$h(t) = \lim_{\Delta t \to 0} \{\frac{1}{\Delta t} Pr\ (t \le T < t + \Delta t | T \ge t)\} \qquad (1)$$

where T designates the time to appearance of the first tumor. Let $P(t)$ designate the probability of a malignant tumor by time t. Then $P(t)$ is related to the hazard function as follows:

$$P(t) = 1 - \exp\{- \int_0^t h\ (s)\ ds\} \qquad (2)$$

For a more detailed discussion of mathematical carcinogenesis models the reader is referred to Whittemore and Keller (1978).

1.1 Assessment of the Effects of Carcinogens on Cancer Development By A Two-Stage Carcinogenesis Model

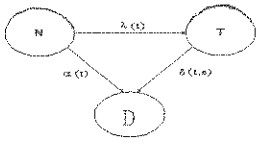
The assumptions required for the MVK two-stage model that will be discussed in this research are as follows. Let $X(t)$ represent the number of normal cells as a homogeneous Poisson process with intensity $\mu_1(t)X(t)$, where $\mu_1(t)$ is the first event rate. In a small time interval, $(t,t+\Delta t)$, an intermediate cell divides into two intermediate cells with probability $\alpha_2\Delta t+o(\Delta t)$; it may die or differentiate with probability $\beta_2\Delta t+o(\Delta t)$. The probability is $o(\Delta t)$ that more than one event will occur.

Several statistical methods have been proposed by scientists to describe the time to appearance and rate of progression of tumors caused by environmental agents. In particular, evaluation of the hazards of chemical carcinogens and their relation to tumor causation and resulting growth rate. The two-stage model for carcinogenesis originally proposed by Moolgavkar and Venzon

(1970), has been recommended as a useful model in environmental risk assessment, (Thorslund et al, 1987; Portier and Bailer, 1989); Moolgavkar and Luebeck, 1990). Besides the consistency of the model with human epidemiological data and animal experimental data, the model is motivated by and based on biological facts, (Moolgavkar and Luebeck, 1990). The objective of this paper is to develop a semi-parametric model to assess the effects of carcinogens on cancer development utilizing the Moolgavkar, Venzon, and Knudson (MVK) two-stage model of carcinogenesis. Some hypothetical initiation-promotion experiments on animals will be developed to test the model.

1.2 Model Development

In practical situations in animal carcinogenicity experiments, animals are exposed to a carcinogen at different dose levels. Initially, any one of the cells in an experimental unit is assumed to be in a normal state, N. Then, it may transfer to the tumor state, T, or to the death state, D. The animal may die naturally or by sacrifice. Autopsy is performed to determine the presence or absence of tumors. In the presence of competing risk in carcinogenesis experiments the exact cause of death is difficult to determine for animals dying from causes other than sacrifices. However, in most cases the tumors of interest are observed at the animals death and assumed to contribute to its mortality. A schematic representation of the model is provided in the following diagram:



In a small time interval, $(t, t + \Delta t)$, the incidence functions of transition between the various states are defined as follows:

(i) The incidence function for natural death without tumor for an animal in state N in time interval $(t, \Delta t)$ is:

$$\alpha(t) = \lim_{\Delta t \to 0} \frac{1}{\Delta t} Pr\ [D \in (t,\ t + \Delta t) | D \geq t,\ T > t] \quad (3)$$

(ii) The incidence function for an animal in state N to develop a tumor in time interval $(t, \Delta t)$ is:

$$\lambda(t) = \lim_{\Delta t \to 0} \frac{1}{\Delta t} Pr\ [T \in (t,\ + \Delta t) | T \geq t,\ D \geq t] \quad (4)$$

(iii) The incidence function for natural death with tumor for an animal in state N in time interval $(t, t + \Delta t)$ is:

$$\delta(t|s) = \lim_{\Delta t \to 0} \frac{1}{\Delta t} Pr\ [D \in (t + \Delta t) | D \geq t,\ T = s \quad (5)$$

The event of death in the interval $(j - 1, j)$ has a probability given by:

$$Pr\ \{D \in (j - 1,\ j),\ T \geq j\} = A\ (j - 1)\ S\ (j) \alpha(j),$$

where an animal starts without tumor in state N. Progression follows to either state D before tumor development at rate $\alpha(t)$, or to state T with tumor development at rate $\lambda(t)$ with subsequent death occurring at rate $\delta(t,s)$.

$$A(j) = \prod_{k=1}^{j} [1 - \alpha(k)]$$

$$S(j) = \exp[-\int_0^j \lambda(x)\ dx] \quad (6)$$

Let the time interval $[0, \Delta t]$ be partitioned into k sub-intervals, covering the time period $[t_0 = 0, t_k = tf]$, where t_0 is the starting time of the experiment. Random variables T, D and η are defined to be time to tumor development, time to natural death and time to potential sacrifice, respectively. The time to sacrifice η is assumed to be independent of both D and T. Furthermore, the competing risk is assumed to be independent of the carcinogenesis process. Let the number of natural cancer-free deaths, natural deaths with cancer, cancer-free sacrifices and number of sacrifices with tumors at dose level $C(=c)$ be designated by d_{1j}, $d_{2j}(c)$, $S_{1j}(c)$ and $S_{2j}(c)$, respectively. P_{1j}, P_{2j}, P_{3j} and P_{4j} are the associated probabilities of $d_{1j}(c)$, $d_{2j}(c)$, $s_{1j}(c)$ and s_{2j}, respectively. The time to cancerous tumor development, T, will be assumed to be a continuous random variable, while D and η are assumed to be discrete random variables. The two-stage model of Moolgavkar-Venzon-Knudson (MVK) will be used as the model for the random variable T. Therefore, the associated incidence function $\lambda_c(t)$, assuming piecewise parameters at time t from the c^{th} level of dose is given as:

(i) The approximated solution for $\lambda_c(t)$ is given by

$$\lambda_c(t) = \mu_1\mu_2 \int_0^t X(s)\ \exp[(\alpha_2 - \beta_2)\ (t - s)] ds \quad (7)$$

(ii) The exact solution for $\lambda_c(t)$ is as follows:

$$\lambda_c(t) = \mu_2 \int_0^t \mu_1(s)\ X(s) \left\{ \exp \int_0^{t-s} [2\alpha_2 \Phi(1,0;u) - (\alpha_2 + \beta_2 + \mu_2)] du \right\} ds \quad (8)$$

In the case of high probability of tumor development the approximated hazard function of Equation (7) is inadequate (Moolgavkar et al, 1988). The use of the exact solution is further supported by Luebeck (personal communication, January 15, 1995). Thus, the exact form of the hazard function in Equation (8) will be used to perform the proposed animal simulation experiments. The parameters of the model are assumed to be piecewise constant. The choice of the MVK model as an unconditional parametric distribution for time to tumor onset and discrete distribution for mortality results in multistate transition probabilities. The parametric modeling for $\lambda_c(t)$ is most commonly used to measure risk assessment and may be more appropriate for quantitative risk assessment than nonparametric models. The cumulative distribution function $F(t;\theta)$ for time to tumor development is given by

$$F\ (t_j;\theta) = 1 - \exp[-\int_0^{t_j} \lambda_c(s)\ ds] \quad (9)$$

The hazard function for natural death without tumor is given by

$$\alpha_{j,c} = Pr\ \{D = t_j | D \geq t_j,\ T > t_j\}.$$

Thus,

$$\hat{\alpha}_{j,c}\ (\theta) = s_{1j}\ [n_c\ S(t_j)\ \hat{A}_{j-1}\ Q_{j-1}]^{-1} \quad (10)$$

where,

$$Q_{j-1} = \prod_{k=1}^{j} (1 - q_{k,c}) \quad \text{and} \quad \hat{A}_{j,c} = \prod_{k=1}^{j} (1 - \hat{\alpha}_{k,c})$$

and $q_{k,c}$ is the probability that an animal is sacrificed at time t_j. The hazard function for natural death with tumor is given by

$$\zeta_j(\theta) = s_{2j}\{n_c Q_{j-1}[\sum_{k=1}^{j} \Delta_k\ (\theta)\ \hat{A}_{k-1} \hat{Z}_{kj-a}]\}^{-1} \quad (11)$$

where

$$\Delta_k(\theta) = F(t_k;\theta) - F(t_{k-1};\theta) \quad \text{and} \quad Z_{kj} = \prod_{m=k}^{j} (1 - \hat{\zeta}_m).$$

For our simulated experiments we assume that the number of normal cells is constant and the animals received various concentrations of dose defined as initiator and promoter. Within the framework of the MVK model, the initiator is an agent that

affects mutation rates, while the promoter is an agent that modifies the kinetics of cell division, (Moolgavkar et al, 1983). To incorporate the effects of initiators and promoters we introduce the following parameterizations. The parameter α_2 is assumed to be 10 per cell per week, (Moolgavkar and Luebeck, 1990). Thus, we have the following parameters: $\alpha_2 = 10.00$,

$\mu_{1c} = c_1 \exp[p_1 \ln(1 + C_I)]$, $\mu_{2c} = c_2 \exp[p_2 \ln(1 + C_I)]$, $\alpha_{2c} - \beta_{2c} = a + b\ln(1 + C_p)$, where C_I is an initiator, C_p is a promoter, and ln is the natural logarithm.

Now we proceed to derive $\lambda_c(t)$ in an instructive approach avoiding a complicated integration. Following Singh et al (1993) we can represent the hazard function in equation (8) for the c^{th} dose level and one initial cell at time u as follows:

$$\lambda_c(t) = 2N_0\mu_{1c}\mu_{2c}\{\exp[\gamma_c(t - t_0)] - 1\}u \qquad (12)$$

$$\{(\gamma_c + \epsilon_c) + (\gamma_c + \epsilon_c)\exp[\gamma_c(t - t_0)]\}^{-1}$$

where

$\gamma_c = [(\alpha_2 + \beta_{2c} + \mu_{2c}) - 4\alpha_{2c}\beta_{2c}]^{\frac{1}{2}}$ and $\epsilon_c = \alpha_2 - \beta_{2c} - \mu_{2c}$.

For this model we suppose that animals are exposed to a carcinogen at constant exposure between t_1 and t_2 ($t_1 < t_2$), and there are no exposure to animals elsewhere. Then we divide the time interval $(0,t)$ into three time intervals. They are $(t_0, t_1]$, $(t_1, t_2]$, and $(t_2, t_3]$ where $t_0 = 0$ and $t_3 = t$. Now we define the probabilities P_{1j}, P_{2j}, P_{3j}, and P_{4j} associated with death without cancer, natural death with cancer, sacrifices without cancer and sacrifices with cancer, respectively, as follows:

(i) The probability of tumor-free natural death without tumors at t_j:

$$P_{1j} = \alpha_{j,c}\, Q_{j-1,c}\, A_{j-1,c}\, S_c(t_j) \qquad (13)$$

(ii) The probability of a tumor-free death by sacrifice t_j:

$$P_{2j} = q_{j,c}\, Q_{j-1,c}\, S_c(t_j)\, A_{j-1,c} \qquad (14)$$

(iii) The probability of natural death with tumors at time t_j:

$$P_{3j} = Q_{j-1,c}\{\Sigma_{k=1}^{j} \Delta_k(\theta) A_{k-1} \delta_j Z_{k,j-1}\} \qquad (15)$$

(iv) The probability of a death by sacrifice with tumors at t_j:

$$P_{4j} = q_{j,c}\, Q\{\Sigma_{k=1}^{j} \Delta_k(\theta)\, \delta_j\, Z_{k,j-1}\} \qquad (16)$$

1.3 Case-Control Model for Survival/Sacrifice Experiments

In this section we will consider an experiment with two groups. One group is treated with a carcinogen and the other group is left untreated. We also assumed that the tumor is irreversible and that all animals are initially free of cancer. The random variables T, D, and η are defined to be the time to tumor onset or death without tumor, the time to natural death, and the time to sacrifice, respectively. Let $t_1 < \ldots < t_I$ to be the I distinct observed times of death in treatment and control groups. Let d_{1zi} and d_{2zi} denote the numbers of animal naturally dead without tumors and with tumors in group z at time t_i, respectively. Let s_{1zi} and s_{2zi} denote the numbers of animals sacrificed without tumors and with tumors in group z at time t_i respectively. Let $\delta(t)$ and $Z(t)$ be tumor and group indicator variables, respectively, where

$\delta(t) = 1$ if a tumor has occurred by time t
$= 0$ otherwise

$Z(t) = 1$ if an animal is a member of treated group
$= 0$ otherwise

Then the hazard function of tumor or set for group z at time t is given by

$$\lambda_z(t) = \lim_{\Delta t \to 0} \frac{1}{\Delta t} P[T \in [t, t + \Delta t) | T \geq t, \delta(t) = 1, Z = z],$$

the hazard function of death among live animals free of tumors in group z is given by

$$\alpha_z(t) = \lim_{\Delta t \to 0} \frac{1}{\Delta t} P[T \in [t, t + \Delta t) | T \geq t, \delta(t) = 1, Z = z],$$

the hazard function of death for an animal with tumor in group z is given by

$$\beta_z(t) = \lim_{\Delta t \to 0} \frac{1}{\Delta t} P[D \in [t, t + \Delta t) | D \geq t\ \delta(t) = 1, Z = z].$$

and the hazard of overall death among live animals in group z is

$$\varphi_z(t) = \lim_{\Delta t \to 0} \frac{1}{\Delta T} P[D \in [t, t + \Delta t) | D \geq t, Z = z]$$

Since the distribution of T can have both discrete and continuous terms, Kalbfleisch and Prentice (1980) presented the overall survivor function to time t without developing a tumor as:

$$P(T > t | Z = z) = \exp\{-\int_0^t \lambda_z(s)ds\} \prod_{t_i \leq t} (1 - \alpha_{zi})$$

where,

$$\alpha_{zi} = P(T = t_i, \delta(T) = 0 | T \geq t_i, Z = z)$$

and λ_z is the hazard function for the continuous part. Within the framework of our proposed model we assumed the MVK model for $\lambda_z(t)$ and have no parametric restriction on $\alpha_z(t)$, and $\varphi_z(t)$. Following the argument of Portier and Dinse (1987), the likelihood function is proportional to the product of the following terms over all animals.

The probability of an animal being sacrificed in group z at time t_i and tumor-free is as follows:

$$P(D > t_i, \delta(t_i) = 0 | Z = z) = P(T > t_i | Z = z)$$

and the contribution of this animal to the likelihood is

$$S_z(t_i) \prod_{j=1}^{i} (1 - \alpha_{zj})$$

where,

$$S_z(t_i) = \exp\{-\int_0^{t} \lambda_z(s)\, ds\}.$$

The probability that an animal in group z is sacrificed at time t_i and has a tumor is as follows:

$$P(D > t_i | Z = z) - P(D > t_i, \delta(t_i) = 0 | Z = z)$$

and its contribution to the likelihood is

$$\prod_{j=1}^{i} (1 - \varphi_{zj}) - S_z(t_i) \prod_{j=1}^{i} (1 - \alpha_{zj})$$

where,

$$\varphi_{zi} = P(D = t_i | D \geq t_i, Z = z)$$

The contribution to the likelihood for an animal in group z and died naturally without tumor at time t_i is defined as follows

$$\alpha_{zi} S_z(t_i) \prod_{j=1}^{i-1} (1 - \alpha_{zj})$$

Similarly, the contribution to the likelihood for an animal in group z and died naturally with tumor at time t_i is given by

$$\varphi_{zi} \prod_{j=1}^{i-1} (1 - \varphi_{zj}) - \alpha_{zj} S_z(t_i) \prod_{j=1}^{i-1} (1 - \alpha_{zj})$$

Because of restrictions on the number of pages we will not further discuss the case-control model for survival/sacrifice experiments. The simulation results for this model will be published somewhere else.

1.4 Parameter Estimation

Let $\hat{\alpha}_{j,c}(\theta)$, $\hat{\zeta}_{j,c}$, $\hat{\lambda}_c(t_j)$ and $\hat{S}_c(t_j)$ be the maximum likelihood estimates of $\alpha_{j,c}(\theta)$, $\delta_{j,c}$, $\lambda_c(t_j)$ and $S_c(t_j)$ respectively. The functions $\alpha_{j,c}(\theta)$, $\delta_{j,c}$ in equations (10) and (11), respectively, are functions of the parameters and the value of d_{1j} and d_{2j}. Accordingly, the resulting log likelihood function under a Markov model is a function of the parameters and the data. The log likelihood is given as follows:

$$\log l = \sum_{j=1}^{n_c} s_{1j} \ln \{A_j S_c(t_j)\} + s_{2j} \ln\{\sum_{k=1}^{j} \Delta_k(\theta) Z_k(\theta) A_{k-1}\} + \text{constant} \quad (17)$$

where n_c is the total number of animals for c^{th} dose group in the study and the constant denotes those terms which are constant with respect to θ.

The likelihood equation (17) cannot be solved in closed form. Thus, a numerical method is suggested to find the maximum likelihood estimators for the parameters. There are many numerical methods available to estimate θ numerically. In this study we used the EM algorithm discussed by Dempster et al, (1977) to estimate the parameters. The name EM follows from the fact that each iteration consists of two consecutive steps: expectation and maximization. This algorithm is easy to program and provides a simple method of estimation. The simplicity of the calculations provides an inexpensively executed estimation procedure in spite of the fact that in general, many iterations are required.

In the case where an experiment has multiple dose levels more notations will be introduced. Let c_k, k ($k = 1, \ldots, K$), designate the dose level given to an animal dying naturally from any cause and at any tumor state in dose group k. Let n_c be the total number of animals in the beginning of the experiment for the c^{th} dose level. Then, for the case where multiple dose levels are used, the maximum likelihood estimate for the model parameters follows from the method of a single dose level.

1.5 Simulations

Now, we discuss the procedures and results of initiation-promotion simulation experiments. The experiments can be summarized as follows:

1. Random samples were generated within the framework of semi-parametric model.
2. The animals were observed until time of death either by natural causes or by sacrifice.
3. The presence or absence of tumors was determined by an autopsy following the death or sacrifice of an animal.
4. Incidence rates computed by the model under carcinogens affecting the parameters were compared.

To illustrate the effects of carcinogens on cancer development, n simulated animals were subjected to a carcinogen and followed over time. The time intervals were divided into three subintervals. Namely, subinterval before, subinterval during and subinterval after the exposure. Note that the parameters of the model are functions of the concentrations (c). The concentrations of the initiator and promoter are chosen as $C_p = 90, 40, 60, 100$ and $C_I = 0, 40, 60, 100$. We assumed the number of normal cells is constant with an initial value $N = 10^6$. The parameter α_2 is known to be 10 per cell per week (Moolgavkar and Luebeck, 1989), and the mutation rates take the value $\mu_1 = \mu_2 = 10^{-6}$.

Various experimental situations were considered. In experiment I there is no initiation or promotion effect on any of the parameters. Initiation and promotion doses were introduced to affect μ_1 and $(\alpha_2 - \beta_2)$, respectively, in experiment II. In experiment III the effects are on μ_2 and $(\alpha_2 - \beta_2)$. For experiment IV the affects of initiator were on μ_1 and μ_2 and the effects of promoter were on $(\alpha_2 - \beta_2)$. Promoter effects were applied on $(\alpha_2 - \beta_2)$ in experiment V. Finally, in experiment VI we introduced initiation doses which affected mutation rates μ_1 and μ_2.

1.6 Summary of Simulations

Numerical results of initiation-promotion experiments for various situations are presented in figures 2-9. These survival/sacrifice experiments were conducted to study the effects of carcinogens in a simulated mice experiment. The mice in these experiments were exposed to a carcinogen from the time of weaning until their death. A random number of mice were sacrificed weekly. For each mouse in these experiments the time of death, mode of death (naturally or by sacrifice) and presence of tumors at the time of death were given. Each mouse in the experiment was exposed to various concentrations of the carcinogen as initiator and promoter in the amount of $C_p = 0, 40, 60, 100$ and $C_I = 0, 40, 60, 100$.

In Experiment III, where the carcinogen effects μ_2 and $(\alpha_2 - \beta_2)$ only, the incidence rates were relatively small. The incidence rates changed slightly with the increase in the amount of the initiator or the promoters. Within the framework of the model, in this experiment there was no effects applied to μ_1, the mutation rate of the normal cells. And accordingly, we might have only a small number of intermediate cells generated from normal cells. In this experiment we may conclude that the absence of initiator effect on μ_1 is slowing the carcinogenicity in mice.

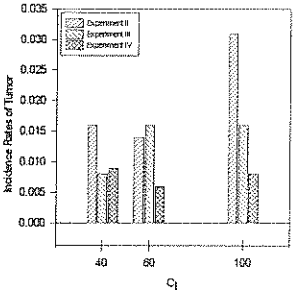
The incidence rates were higher in mice when the carcinogens are affecting the mutation rates μ_1 and μ_2 and cell division rate $(\alpha_2 - \beta_2)$ as in experiment IV or the normal cell mutation rate μ_2 and cell division rate $(\alpha_2 - \beta_2)$ as in experiment II. In these experiments the incidence rates increased as we increased the concentrations of initiator and/or promoter. For both experiments the effects on the dose-response relationships were small when $C_P = 0$ *and* $C_I = 40, 60,$ *or* 100.

Graphical comparisons of the expected incidence rates for experiments II, III, and IV are illustrated in graphs A-G. The graphs show the expected incidence rates for given C_P and different concentrations of C_I and for given C_I and different concentrations of C_P. In these graphs for any amount of C_P we observe that the incidence rates increase considerably as the amount of C_I increases. Whereas, the incidence rates do not change much when C_P has the concentrations $40, 60,$ and 100 for any given dose of C_I. When $C_P = 0$ the incidence rates are minimal. These graphs suggest that the initiator affects the dose-response relationship if it is followed by a considerable dose of promoter. The initiator may not cause cancer if there is no promoter affecting the cell net proliferation rate $(\alpha_2 - \beta_2)$. From

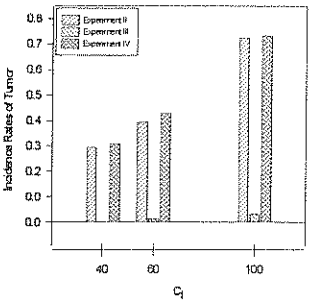
these experiments we may conclude that the most important factors for carcinogenicity are the mutation rates μ_1 and μ_2 and cell division rates.

We observed little effects of the carcinogen on the dose response-relationship in experiments V and VI. Note that in experiment V the carcinogen introduced was a promoter to affect the kinetics of cell division $(\alpha_2 - \beta_2)$ and introduced in experiment V as an initiator to affect the mutation rates μ_1 and μ_2 of normal and intermediate cells.

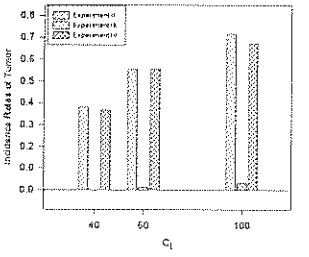
(Figure A)
$C_p = 0$



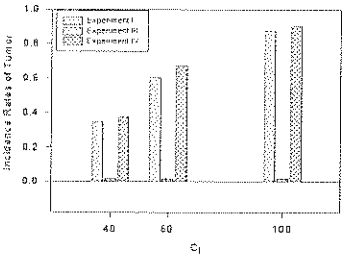
(Figure B)
$C_p = 40$



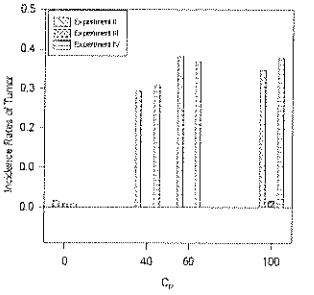
(FigureC)
$C_p = 60$



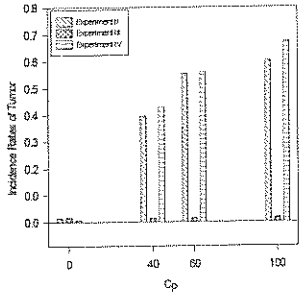
(Figure D)
$C_p = 100$



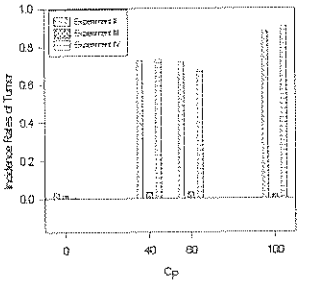
(Figure E)
$C_I = 40$



(Figure F)
$C_t = 60$



(Figure G)
$C_t = 100$



1.7 Acknowledgement

The authors wish to thank Ms. Debbie DePew for her patience and excellent typing.

1.8 References

Armitage, P. and Doll, R., Stochastic models for carcinogenesis: In proceedings of the Fourth Berkeley Symposium on mathematical statistics and probability, J. Noyman (ed), Berkeley and Los Angles, *University of California Press*, (4), 19-38, 1961.

Dempster, A. P., Laird, N. M., and Rubin, D. B., Maximum likelihood estimation from incomplete data via the EM algorithm (with discussion), *J. R. Statit. Soc. B*, (39), 1-38, 1977.

Kalbflisch, J. D. and Prentice, R. L., The statistical analysis of failure time data, New York, John Wiley and Sons, 1980.

Moolgavkar, S. H., Model for human carcinogenesis: Actions of environmental agents, *Environmental Health Perspectives*, (50), 285-291, 1983.

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

Moolgavkar, S. H. and Knudson, A. G., Jr., Mutation and cancer: A model for human carcinogenesis. *Journal of National Cancer Institute*, (55), 1037-1052, 1981.

Moolgavkar, S. H. and Venzon, D. J., Two-event models for carcinogenesis: Incidence curves for childhood and adult tumor, *Mathematical Biosciences*, (47), 55-77, 1979.

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

Moolgavkar, S. H. and Luebeck, G., Multistage carcinogenesis: Population-based model for colon cancer, *Journal of National Cancer Institute*, (84), 610-618, 1992.

Moolgavkar, S. H. and Venzon, D. J., Two-event model for carcinogenesis: Incidence curves for childhood and adult tumors, *Mathematical Biosciences*, (47), 55-77, 1979.

Portier, C. J. and Bailer, A. J., Two-stage models of carcinogenesis for historical control animals from National Toxicology Program, *Fundamental and Applied Toxicology*, (12), 731-737, 1989.

Portier, C. J. and Dinse, G. E., Semi-parametric analysis of tumor incidence rates in survival/sacrifice experiments, *Biometrics*, (43), 107-114, 1987.

Singh, K. P., Bartolucci, A. A., and Katholi, C. R., Statistical procedures for assessing risks of environmental agents, proceedings of the International Congress on modeling and simulation, McAleer, M. and Jackeman, A. (Eds), the University of Western Australia, (4), 1735-1742, 1993.

Thorslund, T. W., Brown, C. C., and Chrnley, G., Biologically motivated cancer risk models, *Risk Analysis*, (7), 109-119, 1987.

Whittemore, A. S. and Keller, J. B., Quantitative theories of carcinogenesis, *SIAM Review*, (20), 1-30. 1978.