Abstract

DONNA G. MARTIN. Application of a Generalized State-Vector Model for Radiation-Induced Cellular Transformation to Multiple *In Vivo* Exposures. (Under The Direction of Dr. DOUGLAS J. CRAWFORD-BROWN)

A generalized state vector model for radiation induced cellular transformation is adapted for use in multiple *in vivo* chemical and radiation exposures. The model was designed to describe the process of radiation carcinogenesis and thus provides for initiation, promotion, and progression as well as cell killing and repair. The model has been modified to describe the combined effects of chemical and radiation induced cell transformation.

Benzo(a)pyrene and polonium-210 markers for environmental tobacco smoke and radon, respectively, were chosen for this study because it is very possible that they will be present together in a variety of indoor environments. The data chosen to test the model come from an experiment by McGandy et al. (1974) in which Syrian golden hamsters were exposed both simultaneously and sequentially to polonium-210 and benzo(a)pyrene.

The results demonstrate that the proposed model can correctly predict that BaP synergism occurs more strongly with high doses of alpha radiation. In addition, the model correctly predicts the increase in tumors found at exposure to low doses of alpha radiation followed by exposure to BaP.

Acknowledgements

I would like to thank my advisor, Dr. Crawford-Brown, for his support and encouragement throughout this project. I would also like to thank my readers, Dr. Ball and Dr. Turner, for their time and valuable insight into the final product.

And of course-- many thanks to my family and friends whose unending patience and support made the completion of this project possible.

Table of Contents

List of Figures

List of Tables

Introduction

Thousands of Superfund hazardous waste sites containing complicated mixtures of chemicals have been identified nationwide. Although primarily sites contaminated by the chemical and petroleum industries, some of the sites are the result of transportation spills or once were municipal landfills now marred by the accumulation of pesticides and household cleaning solvents (USEPA 1987). Although many of the sites have similar chemical mixtures, they are not identical. The chemical mixtures may, however, cause a similar mixture of cellular and other health effects. The same thing can be said of environments with ambient or indoor air contamination such as might arise from environmental tobacco smoke. Although each environment is unique, the mixture of pollutants in the air may cause similar types of effects.

Presently, EPA supports the use of an additive model for the evaluation of the risk to public health from Superfund sites. As instructed in the Superfund Public Health Evaluation Manual (1986), the risk assessor identifies indicator chemicals, pathways and magnitude of exposure. Then, in order to determine the risk at the site, the risk from each individual chemical is modeled amd summed to estimate the total potential risk at the site. In this

approach, no provision is made for the interaction of chemicals at a site. (USEPA 1986)

For an additive model to be correct, all contaminants would have to exert their effect on the same stage of cell transformation or development or there might be entirely different routes to the same effect (cancer). Studies have shown that this does not occur (NCRP #96). Not all exposures cause the same type of cellular damage. Chemicals acting as either initiators or promoters or both can cause a wide range of cellular and organ effects. These cellular effects include but are not limited to: genetic damage, hyperplasia, DNA adduct formation, or cell death. Chemicals can also affect the organs by altering biokinetics or dosimetry. Furthermore, many chemicals require metabolism before exerting their effect.(Farber 1981)

Chemicals can also modify the rate constant of another pollutant in one of several possible ways. For example, a contaminant may interact with existing chemicals and modify the existing chemical before it reaches the cell. Also, a previous chemical or radiation exposure may alter the metabolic properties of the cell, causing the cell to react differently (not normally) to a second exposure.

The generalized state-vector model presented by Crawford-Brown and Hofmann (1990a) considers the interaction of these effects in predicting a cancer risk. Their model asserts that the underlying theory for the multistage model

for the development of cancer, first introduced by Nordling (1953) and later elaborated on by Armitage and Doll (1954), is valid in form if not in the mathematical details. Essentially, the multistage model proposes that a normal cell is transformed by initiation, promotion and progression through several distinct states or stages before eventually reaching a final state of uncontrolled growth. The difference between the multistage model and that of Crawford-Brown and Hofmann is that the multistage model posits transition rate constants between states, while the latter model replaces rate constants by explicit changes in the structural features of a cellular community.

The model proposed by Crawford-Brown and Hofmann (1990a) has been tested against *in vitro* studies for radiation and shown to provide good fits to previously unexplained data. *In vitro* studies, however, are limited. Specifically, they do not take into account metabolic activity, often a factor in chemical carcinogenesis. They also do not include the kinetics of the growth of tumors in the body.

This raises the question-- Can this model be applied to multiple exposures *in vivo*? The dose-response of a chemical is dependent on what state the cell is in at the time of exposure. In *in vivo* studies this could interfere with the predictive ability of the state-vector model and must be incorporated into the model.

Contaminants Chosen for Theoretical Study

Benzo(a)pyrene (BaP) and polonium-210 (Po-210) markers for environmental tobacco smoke (ETS) and radon, respectively, were chosen for this study because it is very possible that they will be present together in a variety of indoor air environments. In addition, these two contaminants exhibit behaviors which are applicable to the model. BaP is a carcinogen which acts both as an initiator and a promoter and causes cytoxicity and hyperplasia. Po-210, a radon decay product, is an alpha particle emitter and thus exhibits the behaviors characteristic of alpha radiation.

The Presence of These Contaminants in Indoor Air

Benzo(a)pyrene

Over 3800 compounds have been identified in the three phases of ETS: sidestream smoke, mainstream smoke and gases that diffuse out from the cigarette while smoking. All three mixtures contain both a particulate and vapor phase. (NRC 1986) BaP has been identified as one of the primary components of the particulate phase of ETS (NRC 1986).

Levels of ETS in the home environment have been quantified. Most of the research has been done on the respirable particle faction of cigarette smoke. Spengler et al. (1981), sampling 80 homes, found that smoking 1 pack of cigarettes a day contributes approximately 20 ug/m^3 to 24-

hour indoor particle concentrations. The 24-hour national ambient air quality standard is 260 ug/m^3. Moschandreas et al. (1987) observed that this level would be exceeded in a house with two or more smokers. Concentrations, in public buildings, bars and restaurants have also been quantified. Repace and Lowry (1980) report levels up to 700 ug/m^3 in bars. Levels of ETS usually peak at the time of smoking and are dependent on the number of smokers and on the type of ventilation.

It is difficult to predict what fraction of these particulates is Bap. Ventilation systems, depending on their efficency and sophistication, may filter out the particulate fraction of ETS which contains BaP. (NRC 1986) It is clear, however, that Bap is present in the air of homes contaminated by environmental tobacco smoke.

Polonium 210

Although it is not usually referred to as a radon daughter (short-lived solid isotopes of Rn-222) primarily because of its 138 day half life, Po-210 is also part of the radon decay chain. It, like Po-214 and Po-218, emits alpha particles during decay. Previous studies have demonstrated the ability of alpha particles to induce tumors in vivo (NAS 1988), thus, polonium-210 exhibits behavior applicable to this study.

Polonium 210 has also been found in tobacco smoke. The parent isotope for Po-210, lead 210, can be detected in ETS in the form of "hot" particles (McGandy et al. 1974) which heavily irradiate small volumes of the lung.

The purpose and scope of this study

The purpose of this study is to determine if the state-vector model is a good predictor of the effect of multiple chemical exposure in vivo. The data used to test the model in vivo was generated in experiments performed and reported by McGandy et al. (1974) and Little et al. (1978). In this experiment (described in more detail later), Syrian golden hamsters were exposed to Po-210 and BaP both simultaneously and sequentially via intratracheal instillation. Upon death or sacrifice (when moribund), the animals were examined histopathologically for the formation of lung tumors. The results of all the experiments will be used simultaneously to test the coherence of this model to observed in vivo data.

The study design includes: First, the general model is described with a discussion of the assumptions and the theoretical solution. Then, the experimental data are presented and the necessary parameters are calculated. These parameters and the experimental exposure levels are then applied to the model and the results observed. Finally, there is a discussion of the applications of this model in environmental management.

The Model

A Brief Review of Carcinogenesis

Although the exact mechanism of carcinogenesis is far from being understood, it is generally accepted that the process involves three basic sequential events; initiation, promotion and progression. Each event has its own characteristics and pathways which lead ultimately to a state of uncontrolled cell growth and the death of an organism.

The process of initiation involves several stages. When cells are exposed to chemical insults, the first stage is biochemical, can be repaired, and is assumed to involve one damage event. It is thought that the chemical forms an electrophilic reactant (often after the chemical has been metabolized) which enables it to bind to and alter components, primarily DNA, in the cell. It is not clear whether the alteration is a small structural or regulatory mutation or a complex rearrangement of large parts of the DNA. (Pitot 1986) With radiation exposure, DNA damage occurs as two, not one, distinct events (Crawford-Brown and Hofmann 1990a; Lloyd et al. 1979) and (for alpha radiation) involves direct breakage of DNA by the deposited energy. Both radiation and chemical damage are followed by cell proliferation which fixes the damage. The ultimate effect of initiation by both chemicals and radiation, is generally

a permanent alteration of some sort in the DNA, RNA, protein or other cellular components. (Farber 1981)

It is not certain what characterizes an initiated cell. These cells in tissues in the skin and colon appear to have lost some ability to program or control growth (Farber 1981), while some initiated liver cells demonstrate an "acquired resistance to the inhibitory effects of carcinogens on cell proliferation" (Farber 1981). It is clear, however, that the number of initiated cells is dependent on dose, a dose that exhibits no threshold (Pitot 1986).

Initiated cells are subsequently stimulated to proliferate to neoplastic lesions. The exact mechanism of this clonal expansion stage is unknown.(Farber 1981) Unlike the process of initiation, promotion to a neoplasm demonstrates a threshold effect and a maximum response. Promotion can also be affected by such factors as diet, environment or hormones (Pitot 1986).

Promotion to a neoplasm is followed by progression. Progression has generally been "characterized by demonstrable changes associated with increased growth rate, increased invasiveness, metastases, and alterations in biochemical and morphologic characteristics of the neoplasm" (Pitot 1986). Like promotion, the mechanism of progression is unclear. It is proposed, however, that the changes seen in progression are often correlated with aneuploidy, a

change in the number of chromosomes in the cells.(Pitot 1986)

A History of State-Vector Models

Scott and Ainsworth (1980) were the first to employ a state-vector model to describe the multistage process of carcinogenesis. Building on an idea proposed by Armitage and Doll (1954) and later elaborated on by Moolgavkar (1978), Moolgavkar and Knudson (1980) and others, Scott and Ainsworth postulated that the development of cancer involved the passing of a cell through several successive stages or states to the ultimate state of uncontrolled cell growth. State-vector models can be used to calculate the fraction of cells in each state or stage. Probabilities of transitions between states (either as a function of dose or dose-rate) are determined from experimental data.

The model used in this study is an adaptation of a generalized state-vector model for radiation-induced cell transformation proposed by Crawford-Brown and Hofmann (1990a). The model was designed to describe the process of radiation carcinogenesis and thus includes initiation, promotion, and progression as well as cell killing and repair. The model has been adapted to describe the combined effects of chemical and radiation -induced cell transformation.

General Features of the Model

The model, a modification of the model for radiation induced cellular transformation described and tested in vitro by Crawford-Brown and Hofmann, is shown in figure 2.1.

State 0 represents cells in the "normal" state. These cells have not as yet experienced a radiation or chemical insult which would result in cellular damage.

Cells in State 1 have experienced specific radiation induced DNA damage such as a strand break. Cells exposed to chemicals, instead of radiation, skip this state and go directly to State 2. Cells that have incurred this damage can undergo repair and revert back to state 0.

A cell reaches State 2 when it experiences a second, radiation induced DNA damage (probably another strand break). This damage is less specific than that required to move from state 0 to state 1, and it is thought to be necessary for the two radiation-induced breaks to interact for initiation to occur (Crawford-Brown and Hofmann 1990a). A cell can also reach this state by incurring chemically induced damage to the DNA, RNA or other cellular components. These cells can also undergo repair and revert back to state 0 (for BaP) or state 1 (for radiation).

Cells in State 3 have undergone division subsequent to incurring DNA damage in State Two. According to Crawford Brown and Hofmann (1990a) this division must occur for a cell to reach state 3, and it occurs with a fixed

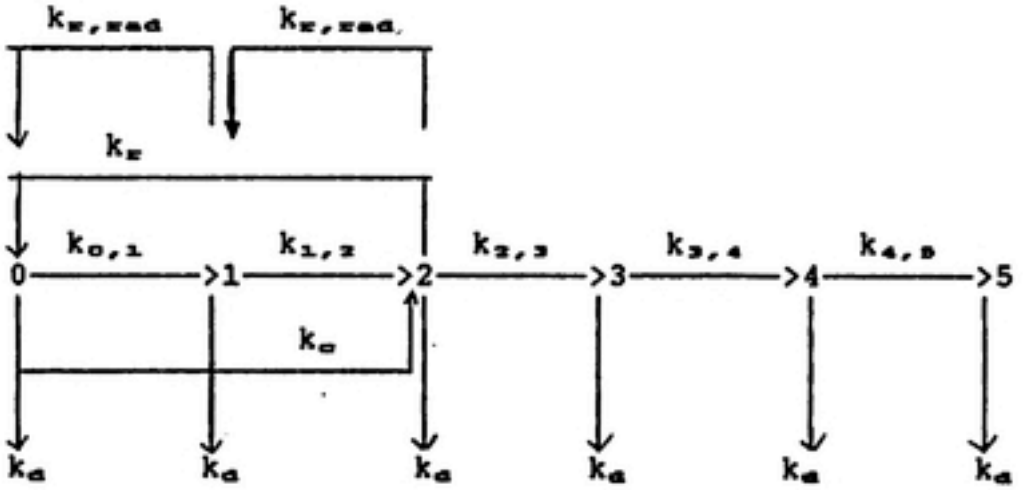


Figure 2.1: The General Model

probability and prevents regression of the cell back to previous states.

By the time cells reach State 4, they have undergone promotion to a neoplastic lesion. This is proposed to occur through the loss of cellular intercommunication, at times referred to as contact inhibition. The concept of contact inhibition removal, although vague, supposes a cell surrounded by dead cells loses its ability to communicate and consequently enters into a state of increased growth. While localized cell death may result in the loss of communication, there clearly are other biochemical routes for this change. As a result, chemicals and radiation may produce the change by different pathways.

Cells in State 5, the final state, have progressed to a stage of uncontrolled cell growth. It is not known how long it takes, or what triggers, a neoplastic lesion in state four to progress to this state. Some idea of the length of time presumably may be obtained by observing the latency period, which may be decades in solid tumors. The latency period may, however, also include the time to promotion.

The transition constant, k_a, represents the rate of removal of a cell from any of the six stages due to radiation or chemical -induced cell death. Its units are in inverse dose (or dose-rate) and its value is obtained from cell survival curves. All chemicals and types of radiation

have different cell survival curves, thus it follows that the cell death transition rate constants obtained from the curves will be unique.

K_r and $k_{r,rad}$ represent the rate of repair of cellular damage caused by chemical and radiation exposure respectively, allowing cells to move out of state two or one and back to state one or zero. The probability of repair is dependent on time and upon the source of damage (radiation or chemical).

The transition constant $k_{0,1}$ represents the transformation of a cell from the zero state to state one. It is quantified by the probability per unit dose that some type of DNA damage occurs. This value, which is in units of inverse dose, is different for each type of radiation.

The radiation-induced transformation of a cell from State 1 to State 2 is represented by $k_{1,2}$. It is the probability per unit dose that a second less specific damage occurs and interacts with the first damage.

It is assumed that chemicals, specifically BaP, exhibit single hit kinetics (see Grimmer et al. 1988) As a result, only one chemically-induced damage event, not two, is required to transform a cell to state 2. In this model the probability of a cell damaged by chemicals reaching state 2 is represented by k_c. It, like $k_{0,1}$ and $k_{1,2}$, is dependent upon the chemical to which the cell is exposed.

Transition $k_{2,3}$ represents the fixed probability that a division related event will occur prior to repair or cell death. It has been demonstrated that the cellular rate of division, and subsequently $k_{2,3}$, is dose dependent (Shuman 1989). The cellular rate of division also depends upon the presence of hyperplastic agents (such as BaP)

$k_{3,4}$ represents the transition from State 3 to State 4. It is the probability that contact inhibition will occur, thus causing the cell to increase its rate of growth to a neoplastic lesion. Because contact inhibition is dependent on cell death (for radiation), a dose dependent event, this transition, like the previous ones, is also dose dependent. For chemicals, the transition is related to hyperplasia and is dependent upon dose-rate.

Finally, $k_{4,5}$ represents the probability of progression to a final state of uncontrolled cell growth.

Identifying The Stages of Promotion and Progression

Although this model represents promotion and progression as separate and distinct stages, in reality it is not this clear cut. Without additional research, it is not possible to discern where promotion ends and progression begins or, which is most likely the case, where the two stages overlap. As a result, these stages are collapsed in the present study, implying that progression occurs with a fixed probability. (Little et al. 1978)

Accepting the Model *In Vivo*

In order to apply this model *in vivo* it must be assumed that *in vitro* neoplastic cell transformation is representative of the mechanism of cancerous transformation *in vivo*. This assumption is supported by the work of Lloyd et al. (1979) in which C3H mice injected with cells transformed *in vitro* by alpha particles developed malignancies. It is assumed here, therefore, that the *in vitro* model may be applied *in vivo* with appropriate changes due to the differences in the kinetics of cellular growth and repair.

Theoretical Solution of the Model

Assumptions Necessary for a Workable Solution

In order to provide a workable solution for this study, several additional assumptions had to be made. They are:

Cellular Environment. For the purposes of this study, it is assumed that there is a constant number of cells in an organ. In addition, there is a constant, steady state concentration of chemical and chemical metabolites throughout the period of exposure.

Dose. The mucous membrane layer of the lung affects the migration of the contaminant to the cellular level. Therefore, in order to simplify the equation, the dose to the organ is considered to be a constant fraction of the amount applied directly to the surface. Furthermore, it is assumed that the spatial pattern of dose rate in the lung does not affect the model.

Cell killing. Cell killing is assumed to occur at a constant rate during the period of exposure and is independent of other transitions in the model. In addition, if a cell makes it to the division stage, the chance of dying does not depend on age of the cell. It will be assumed that BaP does not cause significant cell killing compared to radiation. The effects of cytotoxicity caused by BaP will be included in determined parameter values.

Division and Repair. It is assumed that a cell is no longer able to repair its damage once it has undergone division leading to state three. In addition, repair is assumed to be independent of the first three transitions. (Crawford-Brown and Hofmann 1990a; Shuman 1989) Alpha radiation induced damage does not repair itself at all (Heiber et al. 1987), except when the cell is stimulated by promoting agents (Crawford-Brown and Hofmann 1990b).

Contact Inhibition Removal. It is assumed that every cell surrounded by the correct number of dead cells (n=4) will experience contact inhibition removal. This value was calculated by Crawford-Brown and Hofmann (1990a) for *in vitro* irradiation. For layers of epithelial cells, the total number of neighboring cells is six (Crawford-Brown and Hofmann 1990a, see figure 5.1).

Promotion. It is assumed that there is a background probability of promotion equal to 0.1 (Crawford-Brown and Hofmann 1990a). In addition, it is assumed that growth to the pre-neoplastic stage occurs rapidly with respect to the animals lifetime in the high dose-rate experiments used here. The lag between exposure and promotion is not modeled here, as might be required if temporal hazard functions were used to estimate the effects of competing risk. Latency has not been explicitly considered in this model.

The Solution

Considering the above assumptions, the probability that a cell will reach state 5, a state of uncontrolled cell growth, can be predicted by the following equation:

$$P_c = kN_2(t)(f_r)\left[\frac{(f_{BaP}m + k_\alpha \dot{D}_r)}{(f_{BaP}m + 2k_\alpha \dot{D}_r + k_r)}\right]\left(1 - e^{-(A + k^* f_{BaP} + f_{ci})}\right)$$

where:

k= a normalization constant including $k_{4,5}$, the number of cells at risk and the fraction of divisions leading from state 2 to state 3 (see Crawford-Brown and Hofmann 1990a).

$N_2(t)$= the number of cells in state 2 at time, t

t= time (in weeks)

$f_r = (1 - e^{-k_\alpha \dot{D} T})/ k_\alpha \dot{D} T$,where f_r is the fraction of N_2 cells which survive the radiation after one day

f_{BaP}= the fractional increase in hyperplasia due to exposure to BaP proportional to $\dot{D}_c$

$\dot{D}_c$= the dose-rate of the chemical (in mg/wk)

m= the rate of mitosis in undamaged cells

k_α= the rate constant for alpha radiation induced cell killing

$\dot{D}_r$= the dose-rate of radiation (in rads/wk)

k_r= the rate of repair of radiation induced cell damage

A= the background probability of promotion

k^*= a parameter relating $\dot{D}_c$ to the probability of promotion

f_{ci}= the probability of contact inhibition removal occurring

Derivation of the Solution

In order to obtain the above solution, it is necessary to first solve the differential equations for $N_2(t)$.

Solving for $N_2(t)$

The first part of the equation accounts for cell transformation/initiation, bringing cells into the second state. Solving for $N_2(t)$ requires first solving for the number of cells in the zeroth state.

$$\frac{dN_0(t)}{dt} = k_r N_1(t) - (k_d \dot{D}_r + k_{0,1} \dot{D}_r + k_c \dot{D}_c) N_0(t)$$

where:

$N_0(t)$= the number of cells in the zeroth state at time, t

$N_1(t)$= the number of cells in the first state at time, t

k_r = the repair rate constant

$k_{0,1}$ = the rate constant for radiation induced cell transformation from state 0 to state 1, in $rads^{-1}$

k_d = the cell killing rate constant

k_c = the rate constant for chemical induced cell transformation from state 0 to state 2

$k_r N_1(t)$, the rate of repair back to state 0 is small compared to the transformation frequency, therefore the equation becomes:

$$dN_0(t) + (k_d \dot{D}_r + k_{0,1} \dot{D}_r + k_c \dot{D}_c) N_1(t) = 0$$

solving using Bernoulli's solutions (Kells 1960), yields

$$N_0(t) = N_0 e^{-(k_d \dot{D}_r + k_{0,1} \dot{D}_r + k_c \dot{D}_c)t}$$

Next, it is then necessary to solve for the number of cells in the first state.

$$\frac{dN_1(t)}{dt} = k_{0,1}\dot{D}_r N_0(t) - k_{1,2}\dot{D}_r N_1(t) - k_d\dot{D}_r N_1(t) - k_r N_1(t) - k_c\dot{D}_c N_1(t)$$

where:

$N_2(t)$= the number of cells in the second state at time, t

$k_{1,2}$ = the rate constant for radiation induced transformation from state 1 to state 2, in $rads^{-1}$

Again, solving with the aid of Bernoulli's solutions (Kells 1960)

$$N_1(t)e^{(k_{1,2}\dot{D}_r+k_c\dot{D}_c+k_d\dot{D}_r+k_r)t}$$

$$= \int_0^t k_{0,1}\dot{D}_r N_0(t)\, e^{(k_c\dot{D}_c+k_d\dot{D}_r+k_r+k_{1,2}\dot{D}_r)t}\, dt + N_1$$

$$= k_{0,1}\dot{D}_r N_0 \int_0^t e^{(k_{1,2}\dot{D}_r+k_d\dot{D}_r+k_c\dot{D}_c+k_r)t}\, e^{-(k_d\dot{D}_r+k_{0,1}\dot{D}_r+k_c\dot{D}_c)t}\, dt + N_1$$

$$= k_{0,1}\dot{D}_r N_0 \int_0^t e^{(k_{1,2}\dot{D}_r-k_{0,1}\dot{D}_r+k_r)t}\, dt + N_1$$

Therefore, the number of cells in the first state equals,

$$N_1(t) = \frac{k_{0,1}\dot{D}_r N_0}{k_r+k_{1,2}\dot{D}_r-k_{0,1}\dot{D}_r}\left(e^{-(k_d\dot{D}_r+k_{0,1}\dot{D}_r+k_c\dot{D}_c)t} - e^{-(k_{1,2}\dot{D}_r+k_d\dot{D}_r+k_r+k_c\dot{D}_c)t}\right) + N_1 e^{-(k_{1,2}\dot{D}_r+k_d\dot{D}_r+k_c\dot{D}_c+k_r)t}$$

Finally, the number of cells in the second state can be calculated in the same manner by inserting in the above equations.

$$\frac{dN_2(t)}{dt} = k_{1,2}\dot{D}_r N_1(t) - k_r N_2(t) - k_d\dot{D}_r N_2(t) + k_c\dot{D}_c N_1(t) + k_c\dot{D}_c N_0(t)$$

Because we are only interested in ways into state 2 (pathways out are being accounted for in later terms), the equation becomes ;

$$\frac{dN_2(t)}{dt} = k_{1,2}\dot{D}_x N_1(t) + k_c\dot{D}_c N_0(t) + k_c\dot{D}_c N_1(t)$$

Therefore,

$$N_2(t) = \int_0^t (k_{1,2}\dot{D}_x + k_c\dot{D}_c)\ N_1(t)dt + \int_0^t k_c\dot{D}_c\ N_0(t)dt + N_2$$

$$= (k_c\dot{D}_c N_0) \int_0^t e^{-(k_a\dot{D}_x + k_{0,1}\dot{D}_x + k_c\dot{D}_c)t}\ dt + (k_{1,2}\dot{D}_x + k_c\dot{D}_c) \int_0^t N_1(t)dt + N_2$$

Thus, $N_2(t)$ equals

$$= \frac{(k_c\dot{D}_c N_0)\left[1 - e^{-(k_a\dot{D}_x + k_{0,1}\dot{D}_x + k_c\dot{D}_c)t}\right]}{(k_a\dot{D}_x + k_{0,1}\dot{D}_x + k_c\dot{D}_c)}$$

$$+ \frac{(k_{1,2}\dot{D}_x + k_c\dot{D}_c)(k_{0,1}\dot{D}_x N_0)\left[1 - e^{-(k_a\dot{D}_x + k_{0,1}\dot{D}_x + k_c\dot{D}_c)t}\right]}{(k_x + k_{1,2}\dot{D}_x - k_{0,1}\dot{D}_x)(k_a\dot{D}_x + k_{0,1}\dot{D}_x + k_c\dot{D}_c)}$$

$$- \frac{(k_{1,2}\dot{D}_x + k_c\dot{D}_c)(k_{0,1}\dot{D}_x N_0)\left[1 - e^{-(k_a\dot{D}_x + k_{1,2}\dot{D}_x + k_c\dot{D}_c + k_x)t}\right]}{(k_x + k_{1,2}\dot{D}_x - k_{0,1}\dot{D}_x)(k_a\dot{D}_x + k_{1,2}\dot{D}_x + k_c\dot{D}_c + k_x)}$$

$$+ \frac{(k_{1,2}\dot{D}_x + k_c\dot{D}_c)(N_1)\left[1 - e^{-(k_a\dot{D}_x + k_{1,2}\dot{D}_x + k_c\dot{D}_c + k_x)t}\right]}{(k_a\dot{D}_x + k_{1,2}\dot{D}_x + k_c\dot{D}_c + k_x)}$$

$$+ N_2$$

Solving for $N_3(t)$

The number of cells in state three, $N_3(t)$, is the number of cells transformed into state 2 which have subsequently divided prior to being repaired or killed. To solve for $N_3(t)$, the number of cells in state 2 is multiplied by (1) f_x and (2) the rate of mitosis divided by the sum of the rate of mitosis and the rate of repair and rate of cell killing.

$$\text{i.e. } P_{2,3} = \frac{f_{map}m + k_c\dot{D}_x}{f_{map}m + k_c\dot{D}_x + k_c\dot{D}_x + k_x}$$

It is assumed here that cell killing stimulates division of the remaining cells.

$$N_3(t) \text{ therefore} = N_2(t) * \frac{k_{2,3}\dot{D}_x}{k_x+k_{2,3}\dot{D}_x+k_c\dot{D}_x} * f_x$$

where:

The fraction of cells going from state two to state three equals $k_{2,3}\dot{D}_x/(k_x+k_{2,3}\dot{D}_x+k_c\dot{D}_x)$. That is the fraction of cells which will move to state 3 within one day before being killed or repaired

$k_{2,3}D$ = the fractional rate of mitosis= $f_{map}m + k_c\dot{D}_x$
Therefore the above fraction = $(f_{map}m + k_c\dot{D}_x)/(f_{map}m + 2k_c\dot{D}_x + k_x)$

f_x= the fraction of state 2 cells that survive the remaining radiation after one day

Given t=o at the beginning of exposure and t=T at the end of exposure, the probability of a cell which moves into state 2 at time, t, surviving to time, T,

equals $e^{-0.0167\dot{D}(T-t)}$.

The fraction of cells surviving to time, T

$$= \int_0^T K\, e^{-0.0167\dot{D}(T-t)}\, dt$$

$$= K\, e^{-0.0167\dot{D}T} \int_0^T e^{+0.0167\dot{D}T}\, dt$$

$$= \frac{K\, e^{-0.0167\dot{D}T}\,(e^{0.0167\dot{D}T}-1)}{0.0167\dot{D}T}$$

$$= \frac{K\,(1-e^{-0.0167\dot{D}T})}{0.0167\dot{D}T}$$

but K=1 therefore, $f_x = \dfrac{(1-e^{-0.0167\dot{D}T})}{0.0167\dot{D}T}$

Solving for $N_4(t)$

The probability a cell moves from state 3 to state 4 is dependent on the probability that the cell is surrounded by n dead cells at the moment of a division and the increase in hyperplasia due to chemical exposure. Thus, the number of cells in state 4 equals:

$$N_4(t) = N_3(t) * (1 - e^{-(A + f_{ci} + k^* f_{BaP})})$$

where;

A= background probability of promotion = 0.1 (Crawford-Brown and Hofmann 1990a)

$$f_{ci} = \frac{x!\, f^n\, (1-f)^{x-n}}{(x-n)!\, n!}$$

f= the fraction of cells, surrounding a state 3 cell, which are dead at any moment. In general, $f = (m + k_d \dot{D}_x)/R$.

m= fractional rate of mitosis= 0.01 day^{-1} (Hall 1985)

R= fractional rate of removal= 1 day^{-1} (Crawford-Brown and Hofmann 1990a)

x= total number of neighboring cells

n= number of dead cells required for contact inhibition removal to occur

k^*= a parameter relating hyperplasia and promotion

f_{BaP}= the fractional increase in hyperplasia due to BaP

Solving for $N_5(t)$

Finally, the probability of a cell exposed to both chemical and radiation reaching state 5, a stage of uncontrolled cell growth is equal to

$$P_c = kN_2(t)(f_r)\left[\frac{(f_{BaP}m + k_d\dot{D}_r)}{(f_{BaP}m + 2k_d\dot{D}_r + k_r)}\right]\left(1 - e^{-(A + k^*f_{BaP} + f_{ci})}\right)$$

where:

k= a normalization constant including $k_{4,5}$, the number of cells at risk and the fraction of divisions leading from state 2 to state 3 (see Crawford-Brown and Hofmann 1989).

$N_2(t)$= the number of cells in state 2 at time, t

t= time, in weeks

f_{BaP}= the fractional increase in hyperplasia due to exposure to BaP

m= the rate of mitosis in undamaged organs.

k_d= the rate constant for radiation induced cell killing

$\dot{D}_r$= the dose-rate of radiation in rads/wk

k_r= the rate of repair of chemical induced cell damage

A= the background probability of promotion

k^*= an undetermined parameter as described earlier

f_{ci}= the probability of contact inhibition removal occurring

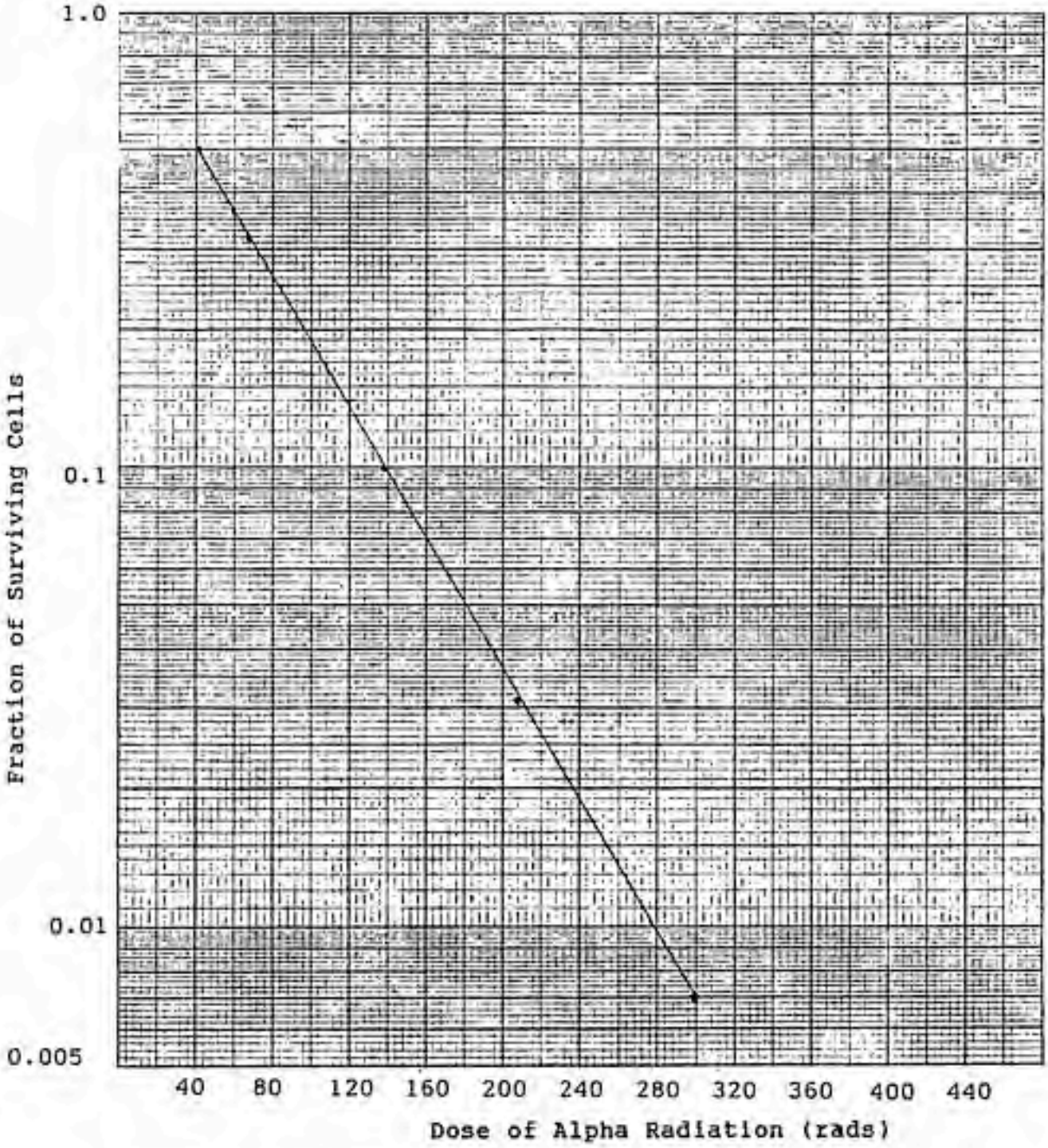
f_r = the fraction of state 2 cells that survive the remaining irradiation after one day

Experimental Data

As discussed in previous chapters, the state-vector model derived for this study allows for the prediction of the probability of a cell exposed to both BaP and Po-210 reaching a stage of uncontrolled cellular growth. Such predictions require the determination of necessary parameter values from experimental data. Alpha-emitting radiation and BaP have been shown to cause initiation, cell transformation, cell killing, hyperplasia, and contact inhibition removal. All of these effects play a significant role in modelling the probability of a cell reaching a stage of uncontrolled cellular growth. In this chapter, observed data from experiments with BaP and Po-210, given both together and alone, will be described, and parameter values necessary to the theoretical model will be calculated.

Cell Survival

The cell survival curve for alpha radiation is taken from an experiment by Lloyd et al. (1979). In this study C3H T1/2, clone 8 cells were irradiated with 5.6 MeV alpha radiation. The resulting cell survival curve shown in figure 4.1 demonstrates that cell killing takes on an exponential form with a mean lethal dose, D_o, of 60 rads (4.4E-06 alphas/cm^2). This yields a survival fraction of



Source: Lloyd et al. 1979

Figure 4.1: Fraction of Surviving Cells vs. Dose of Alpha Radiation

$$S(D) = e^{-k_{\alpha}D}$$

where:

S(D)=surviving fraction at dose, D

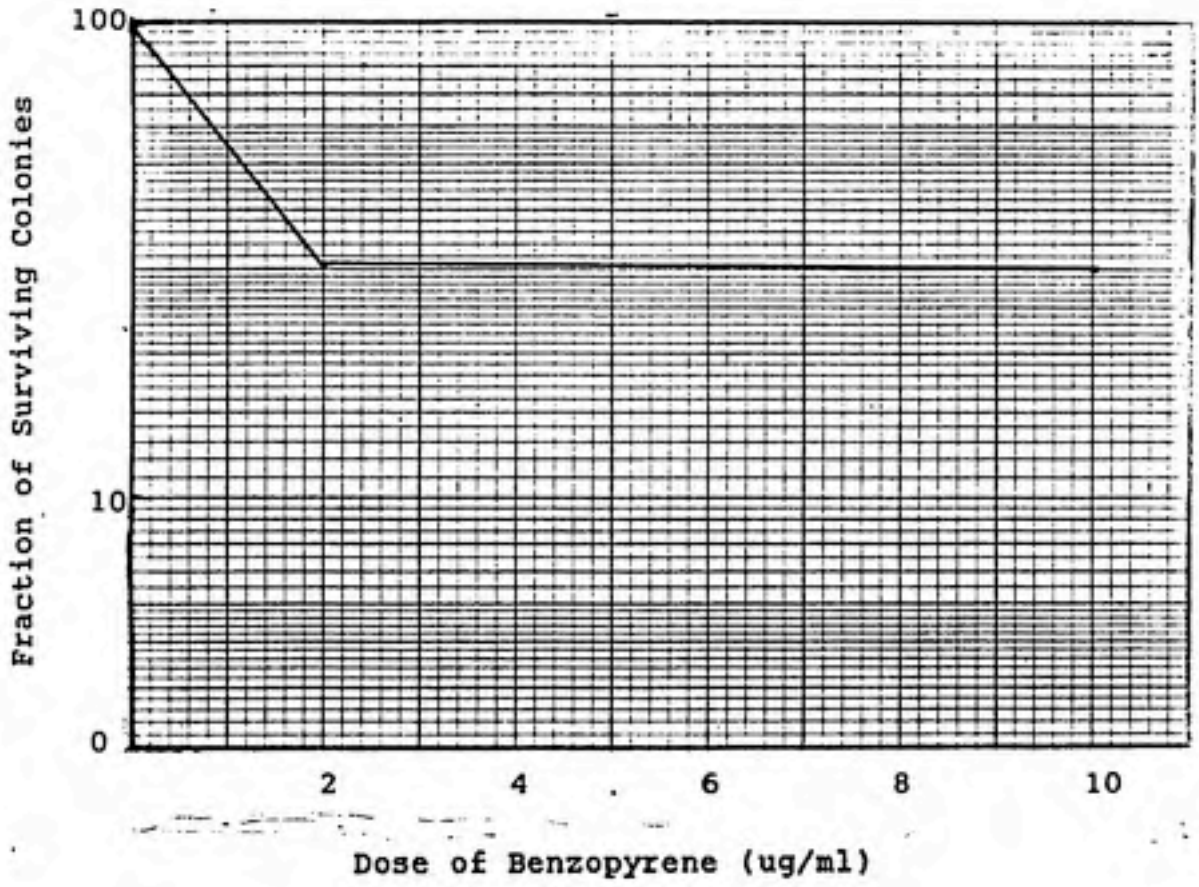
e= natural logarithm

D= dose in rads

k_{α}= cell killing rate constant in $rads^{-1}$

From the data, k_{α} for alpha radiation equals 1/60 rads or 1.67E-02 $rads^{-1}$.

Cytotoxicity data for benzopyrene are provided by Gelboin et al. (1969). Gelboin et al. (1969) exposed normal hamster cells to very low doses of benzopyrene. The results, shown in figure 4.2 as a percentage of surviving colonies in treated plates versus dose of benzopyrene in ug/ml, demonstrate that very low doses will apparently kill a substantial percentage of normal hamster cell colonies. These results conflict with in vivo results reported by McGandy et al. (1974). This study, discussed in greater detail later, found that 7.6 percent of hamsters exposed to a total dose of BaP of 4.5 mg developed frank tumors. Given that the mass of a hamster lung is 0.74 $\pm$ 0.02 gms (Bivan et al. 1987), a dose of 4.5 mg would be equivalent to 6.08E+03 ug/ml. At this dose, Gelboin et al. (1969) would predict very low survival, suggesting no tumors.



Source: Gelboin et al. 1969

Figure 4.2: Cell Survival vs. Dose of Benzopyrene

In order to reconcile the discrepancies between these two experiments, it has been assumed for the purpose of this study that the amount of BaP instilled on the lung surface does not constitute the delivered dose in cells in vivo. It is further assumed that BaP does not cause significant cell killing when compared to alpha radiation, which is a potent cytotoxic agent. The effects of any cytotoxicity will, therefore, be hidden in the determined parameter values for k^* and k_α.

Initiation/ Cell Transformation

In the proposed model, a cell is assumed to be initiated by the time it reaches state 3 (for model and formula see chapter 2).

As discussed previously, radiation induced transformation is thought to be a two step process. The first step, represented in the model as $k_{0,1}$ is the transition between state 0 and state 1 which requires the radiation to induce some type of DNA damage such as a strand break. Crawford-Brown and Hofmann (1990a) have calculated that the value of $k_{0,1}$ is 1.0E-04 times the alpha radiation cell killing rate constant, k_α. This yields a value of $k_{0,1}$ equal to 1.67E-06 rad^{-1}. based on a physical argument focusing on target size.

The $k_{1,2}$ transition, requiring a second less specific DNA insult, has been determined by a curve fit of data by

Crawford-Brown and Hofmann (1990a). They estimate the value of $k_{1,2}$ for alpha radiation to be 0.04 $rads^{-1}$. This value has been adopted for this study.

N_0, N_1, and N_2 represent the number of cells in state 0, state 1, and state 2 before receiving any radiation or chemical exposure. The number of cells, as a proportion of the total number of cells in the organ, has been determined by Crawford-Brown and Hofmann (1990a) to be 0.94, 0.06, and 0.001, for N_0, N_1, and N_2, respectively. These values are referred to as the initial state vector. In the initial state, although they have not as yet received a radiation or chemical insults, cells can be moved from the zeroth state to the second state by background radiation, spontaneous transformations, or other miscellaneous cellular effects.(Crawford-Brown and Hofmann 1990a; Shuman 1989)

These values were determined for the initial <u>in vitro</u> state vector, in the absence of <u>in vivo</u> data. Although there will certainly be differences between the <u>in vitro</u> and the <u>in vivo</u> state vectors, at this time the <u>in vitro</u> values represent the best available estimate and were adopted for this study.

It will be shown later that BaP is only producing a small fraction of initiation above the initial state vector. In the presence of radiation, BaP appears to function primarily as a promoter.

DNA Repair

Damage caused by alpha radiation can not be repaired (Crawford-Brown and Hofmann 1990a) unless stimulated by the presence of strong hyperplastic agents (Crawford-Brown and Hofmann 1990b). DNA damaged (in the form of DNA adducts) by BaP has, however, been shown to undergo repair in hamster cells at a rate of 50% or 0.5 per day (Eastman et al. 1981). For the purpose of this study, repair is assumed to be constant over time. Therefore, k_r equals 0.5 per day for the BaP experiments. The value of k_r for alpha damage in the presence of BaP will be determined during curve fitting.

Promotion

The probability that BaP will cause promotion is characterized for the purpose of this study by its ability to cause hyperplasia. Chouroulinkov et al. (1979) applied BaP (or acetone control) to an area of clipped dorsal skin of 45 day old CDI female mice. The treatment was repeated every other day until three treatments had been completed. The mice were killed eight days after the last treatment and examined histopathologically. The results demonstrate a linear increase in hyperplasia with increasing dose. The results were extrapolated to higher doses for the purpose of this study (see figure 4.3).

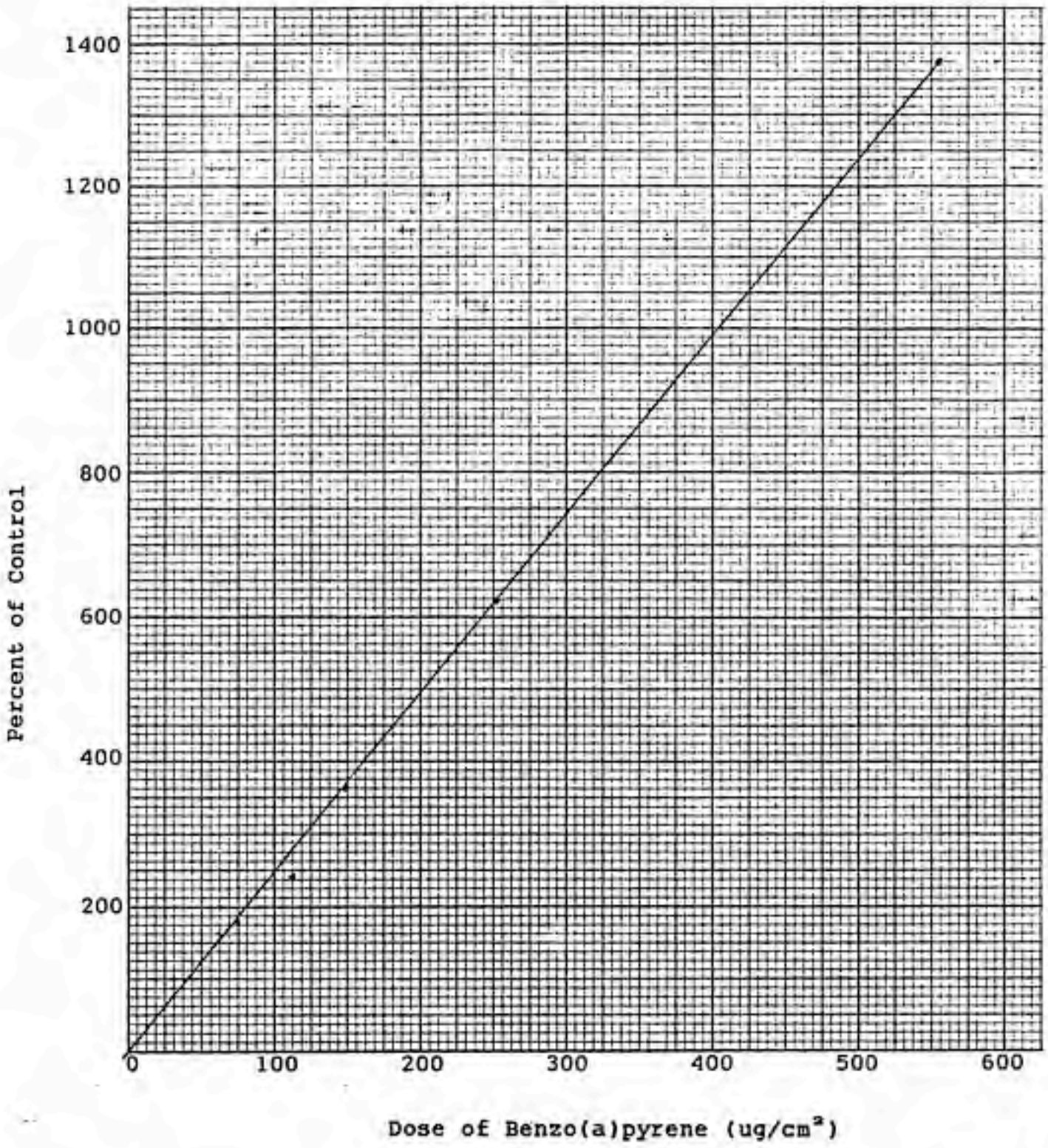


Figure 4.3: Hyperplasia (as % of Control) vs. Dose of Benzo(a)pyrene

Experiment with Benzo(a)pyrene and Polonium-210

McGandy et al.(1974) exposed Syrian golden hamsters both simultaneously and sequentially by intratracheal instillation to Po-210 and BaP. Upon death or sacrifice (when moribund) the lungs and trachea were removed together and examined histopathologically for the formation of tumors.

The dosing regime and percent of animals in each group developing tumors are presented in Table 4.1. McGandy et al. (1974) originally reported exposing the animals to lower radiation doses than appear in this table. This experiment was reported again by Little et al. (1978) with these doses. Personal communication with J. Little (2/22/90) revealed that the doses which appear in table 4.1 are correct.

The next chapter will use this dosing regime to calculate the remaining constants which are necessary in order to solve the model. Experiment 1 in which animals were exposed to 75 rads/15 weeks, allows for the calculation of the rate constant, k. The value of k includes the value of $k_{4,5}$. Given that no frank tumors developed, it is assumed that the cells remain in state 3. Experiments 7 and 9 together provide k^*, the fractional increase in cancer due to promotion from BaP of cells initiated by radiation. Experiments 4 and 5 allow for the calculation of k_{α}, the

Table 4.1: Dosing Regime of Syrian Golden Hamsters

Dosing Regime:		
Group[1]	Treatment [2] (intratracheal instillation)	% w/tumours
Simultaneous Series:		
1 (82)	Po-210 ferric oxide (1.25 nCi x 15 wks) (~75 rads)[3]	12.2
2 (83)	Po-210 ferric oxide (0.25 nCi x 15 wks) (~15 rads)[3]	10.8
3 (66)	BaP ferric oxide (0.3 mg x 15 weeks)	7.6
4 (73)	1 and 3 simultaneously on same ferric oxide particles	34.2
5 (74)	2 and 3 simultaneously on same ferric oxide particles	10.8
Sequential Series:		
6 (65)	Po-210 ferric oxide (0.04 uCi, single instillation)	0.0
7 (74)	Po-210- Saline (0.04 uCi, single instillation)	1.4
8 (72)	6 and 18 wks later BaP ferric oxide (0.3 mg x 7 wks)	18.0
9 (63)	7 and 18 wks later BaP ferric oxide (0.3 mg x 7 wks)	15.9

[1] Number in parentheses is number of animals at risk. Animals that died during the treatment process were not included in prevalence rates.

[2] Treatment periods began when animals were 12 wks of age.

[3] McGandy et al. (1974) orginally reported lower doses. This experiment was reported again by Little et al. (1978) with these doses. Personal communication with J. Little (2/22/90) revealed that the above doses are correct.

rate constant for the repair of DNA damage caused by BaP and radiation. Finally, experiment 3 in which the hamsters were exposed to BaP alone, permits the calculation of k_{α}, the rate at which BaP transforms cells into state 2.

Determination of Parameter Values

The experimental data discussed in the previous chapter, can be used to calculate the remaining constants.

f_{ci}: In order to move into the fourth state, the cell must experience a loss of contact inhibition. Contact inhibition removal occurs when the cell is surrounded by a minimum of n dead cells. The formula for the probability of contact inhibition removal occurring is represented by a binomial distribution and equals:

$$f_{ci} = \frac{x!\, f^n (1-f)^{x-n}}{(x-n)!\, n!}$$

where f, the fraction of dead cells present in the organ = $(m + k_a \dot{D}_x)/R$ (Crawford-Brown and Hofmann 1990b)

For the purpose of this study it is assumed (based on the cell structure of the lung as seen in figure 5.1) that n equals 4 and x (the total number of neighboring cells) equals 6. Given m equals 0.01 day^{-1}, k_a equals 0.0167 rad^{-1} and R equals 1 day^{-1}, f equals 0.0124 and 0.0219 for dose-rates of 1 and 5 rads/wk, respectively. It then follows that for 1 rad/wk f_{ci} equals 3.5E-07 and for 5 rads/wk f_{ci} equals 3.3E-06.

f_{BaP}: For the purposes of this study, f_{BaP}, the fractional increase in hyperplasia, represents the promotional capabilities of BaP. Chouroulinkov

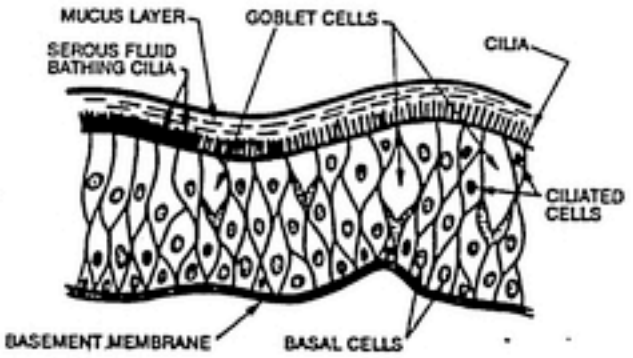


Figure 5.1: Epithelial Cell Layer of the Lung

Source: Cothern and Smith 1987

et al. (1979) demonstrate that in <u>in vivo</u> studies f_{bap} increases with increasing dose. It is assumed that f_{bap} calculated <u>in vivo</u> is proportional to the dose rate of BaP applied <u>in vivo</u> over the entire surface area of the tracheobronchial region. Thus, given that the surface area of the hamster tracheobronchial region is 8.10361 cm^2 (Kennedy et al. 1978), doses of 2.1 mg and 4.5 mg (which both equal a dose-rate of 0.3 mg/wk) would correspond to a f_{bap} of 1.9.

k: k, a normalization constant including $k_{4,5}$, the number of cells at risk and the fraction of divisions leading from state 2 to state 3 can be obtained by solving the following equation for lung tumors in hamsters caused by exposure to alpha radiation only.

$$f = (k)\, N_2(t)\, (f_\alpha) \left[\frac{(k_{2,3}\dot{D}_\alpha)}{(k_\alpha + k_\alpha \dot{D}_\alpha + k_{2,3}\dot{D}_\alpha)}\right](1 - e^{-(A + f_{ci})})$$

where:

f= fraction of animals developing frank tumors

$N_2(t)$= the number of cells in state 2 at time, t

f_α= the fraction of cells in state 2 that survive the remaining radiation after one day

$k_{2,3}$= the transition constant from state 2 to state 3

$\dot{D}_\alpha$= the dose rate of radiation in rads/week

f_{ci}= the probability of contact inhibition removal occurring

A= the background probability of promotion

k_α= the repair rate constant for the repair of alpha radiation

k_α= the rate constant for alpha radiation induced cell killing

Because damage from alpha radiation cannot be repaired, k_r equals 0. Given $k_{2,3}\dot{D}_r$ equals m+ $k_\alpha\dot{D}_r$ (where m= the fractional rate of mitosis) and the above assumptions, the equation becomes

$$f = (k)\, N_2(t)\, (f_r) \left[\frac{(m+k_\alpha\dot{D}_r)}{(m+2k_\alpha\dot{D}_r)}\right] (1-e^{-(A + f_{\alpha i})})$$

Using the data from McGandy et al. (1974) in which 12.2% of animals exposed to 75 rads over 15 weeks developed tumors, k equals 75.8.

k^*: k^*, the fractional increase in cancer in cells initiated by alpha radiation due only to the promotion from BaP, can be calculated by dividing the percent of tumors caused by an acute dose of radiation by the percent of tumors caused by a dose of radiation followed by several doses of BaP. Using the results of McGandy et al. (1974) experiments 7 and 9 the equation for k^* equals:

$$\frac{0.014}{0.124} = \frac{kN_3(t,\ \text{radiation})(1-e^{-(A+f_{\alpha i})})}{kN_3(t,\ \text{radiation})(1-e^{-(A+f_{\alpha i}+k^* f_{BaP})})}$$

where:

0.014= percent of animals with tumors given an acute alpha radiation dose

0.124= percent of tumors caused by promotion by BaP. The percent of tumors initiated by BaP have been subtracted out [15.9-7.6(2.1/4.5)]= 12.4

k= a normalization parameter previously explained

$N_3(t)$= the number of cells in the third state at time, t

A= the background probability of promotion

f_{ci}= the probability of contact inhibition removal occurring

k^*= the fractional increase in hyperplasia due to exposure to BaP of cells initiated by radiation

f_{BaP}= the fractional increase in hyperplasia due to exposure to BaP

Solving the above equation yields a k^* equal to 1.2.

k_c: k_c, the probability per unit dose that a cell damaged by BaP will reach state 2, can be calculated using the results of experiment 3 by McGandy et al. (1974) in which 7.6% of hamsters exposed to a total dose of 4.5 mg BaP only developed tumors. The equation for k_c is as follows:

$$\frac{0.076}{0.069} = \frac{(N_2(t))(f_{BaP}m/f_{BaP}m + k_r)}{(N_2(t))(f_{BaP}m/f_{BaP}m + k_r)}$$

$$= \frac{[(1-e^{-k_c\dot{D}_c t}) + 0.001]\ (0.037)}{0.001}$$

where:

0.069= the approximate percent of tumors that would be calculated by the model if BaP caused no initiation or repair.

$N_2(t)$= the number of cells in state 2 at time, t $N_2(t)$ for 6.9 percent would equal the value of N_2, the fraction of cells in state 2 before receiving any radiation or chemical damage

f_{BaP}= the fractional increase in hyperplasia due to exposure to BaP

m= the typical rate of mitosis= 0.01 day^{-1} (Hall 1985)

k_r= the repair rate constant for repair of DNA damage caused by BaP only= 0.5 day^{-1}

The above equation yields a k_e equal to 0.0065 $dose^{-1}$. The data demonstrate that the fraction of cells in state 2 due to damage by BaP is only 0.00001 above the initial state vector. It appears as if BaP is acting primarily as a promoter, promoting cells that have already reached state 3, and not as an initiator.

k_r: k_r is the repair rate constant for the repair of alpha radiation and chemical damage in the presence of BaP. It is obtained by separately solving McGandy et al. (1974) experiments 4 and 5 for k_r and then calculating the average of the two. The k_r for experiment 4, in which 34.2% of hamsters exposed simultaneously to 75 rads of Po-210 and 4.5 mg of over a period of 15 weeks developed tumors, equals 0.77 wk^{-1}. The k_r for experiment 5, in which 10.8% of hamsters exposed simultaneously to 15 rads of Po-210 and 4.5 mg of over 15 weeks developed tumors, equals 1.9 wk^{-1}. Averaging the two values yields a k_r equal to 1.3 wk^{-1}.

Results

Using the values determined in the previous chapters, the model can now be used to predict the results of all five of the simultaneous exposure experiments reported by McGandy et al. (1974). The parameter values are summarized in table 6.1. The results predicted by the model are as follows.

Experiment 1:

$$P_c = kN_z(t)(f_r)\left[\frac{(f_{sap}m + k_d\dot{D}_r)}{(f_{sap}m + 2k_d\dot{D}_r + k_r)}\right]\left(1 - e^{-(A + k^*f_{sap} + f_{ci})}\right)$$

$$= (75.8)(0.0427)(0.65)(0.57)(0.1) = 12\%$$

Experiment 2:

$P_c = (75.8)(0.0252)(0.84)(0.884)(0.1) = 14\%$

Experiment 3:

$P_c = (75.8)(0.0011)(1)(0.91)(1) = 7.6\%$

Experiment 4:

$P_c = (75.8)(0.0427)(0.14)(0.57)(0.91) = 24\%$

Experiment 5:

$P_c = (75.8)(0.0252)(0.1)(0.884)(0.91) = 15.4\%$

These results are compared with the observed results in table 6.2.

Table 6.1: Parameter Values

Parameter	Value	Source
A	0.1	Crawford-Brown and Hofmann 1990a
$\dot{D}_a$	0.3 mg/wk	McGandy et al. 1974
$\dot{D}_r$	5 rads/wk or 1 rad/wk	Personal Communication with J. Little (2/22/90)
f_{exp}	1.9 $(mg/wk)^{-1}$	Chouroulinkov et al. 1978
f_{ai} (for 1 rad/wk)	3.5E-07	calculated
(for 5 rads/wk)	3.3E-06	calculated
k	75.8	calculated
k^*	1.2 $(mg/wk)^{-1}$	calculated
$k_{0,1}$	1.67E-06 rad^{-1}	Crawford-Brown and Hofmann 1990a
$k_{1,2}$	0.04 rad^{-1}	Crawford-Brown and Hofmann 1990a
k_e	6.5E-03 mg^{-1}	calculated
k_d	0.0167 rad^{-1}	Lloyd et al. 1979
k_r	1.3 wk^{-1}	calculated
$N_2(t)$ (for 1 rad Po-210/wk)	0.0252 cells	calculated
(for 5 rad Po-210/wk)	0.0427 cells	calculated
m	0.01 day^{-1}	Hall 1985
t	15 weeks	McGandy et al. 1974

Table 6.2: Comparison of Observed Results to Predicted Results

Simultaneous Series	Percent of Animals with Tumors	
Experiment #	Observed[1]	Predicted
Experiment 1 Po-210 ferric oxide (1.25 nCi x 15 wks) (~75 rads)	12.2	12
Experiment 2 Po-210 ferric oxide (0.25 nCi x 15 wks) (~15 rads)	10.8	14
Experiment 3 BaP ferric oxide (0.3 mg x 15 weeks)	7.6	7.6
Experiment 4 1 and 3 simultaneously on same ferric oxide particles	34.2	24
Experiment 5 2 and 3 simultaneously on same ferric oxide particles	10.8	15.4

[1] Source: McGandy et al. (1974) and personal communication with J. Little (2/22/90)

Conclusion

The results of this study demonstrate that the proposed model is able to correctly predict that the synergism of BaP and alpha radiation occurs more strongly at high doses of radiation than at low doses. This is clearly shown by comparing the ratio of the result of experiment 4 to that of experiment 1, and the ratio of the result of experiment 5 to the result of experiment 2:

Ratio of Experiment 4 to experiment 1:

Measured: $\frac{\text{experiment 4}}{\text{experiment 1}} = \frac{34.2}{12.2} = 2.8$

Modeled: $\frac{\text{experiment 4}}{\text{experiment 1}} = \frac{24}{12} = 2.0$

Ratio of Experiment 5 to experiment 2:

Measured: $\frac{\text{experiment 5}}{\text{experiment 2}} = \frac{10.8}{10.8} = 1.0$

Modeled: $\frac{\text{experiment 5}}{\text{experiment 2}} = \frac{15.4}{14} = 1.1$

From this comparison, it may be seen that the ratio is higher for experiment 4/1 that for experiment 5/2, both in the case of measurement and model predictions. Thus, in response to McGandy et al.'s statement of uncertainty concerning their results, "It is not clear why this synergistic effect in the simultaneous series appeared to be

associated only with the higher radiation exposure groups", it can be clearly shown that it occurs as a result of the kinetics explicated by the present model.

In addition, the model correctly predicts the ten-fold increase in tumors in animals exposed to low doses of radiation and BaP versus animals exposed to low doses of radiation only. This increase in tumors is due to the saturation of the promotional effect of BaP. Because A (the background rate of promotion) is equal to 0.1, the probability of promotion can only increase ten-fold before the promotional term becomes saturated. In this study, $f_{BaP}k^*$ was shown to be large enough to saturate the promotional term. If BaP were actually causing significant initiation at this dose, there would have been a higher yield of tumors above a ten-fold increase.

The results demonstrate that the model is predictive of observed <u>in vivo</u> results in the form presented and with these two contaminants. This result does not, however, confirm that the model would be predictive with other contaminants or in other environments. For the purpose of this study, BaP, a chemical which has been suggested to exhibit single hit kinetics in the initiation phase of carcinogenesis, served as the model for chemical exposures. If a chemical were shown to require more than one initiation step, the form of the model would have to be adapted to account for this different information. In particular

additional stages or a non-linear model would be required.

The model, as presented, does not account for the temporal appearance of effects following exposure or temporal changes in the relationship between exposure and dose-rate. Specifically, it was assumed here that the amount of chemical or radiation that reached the cell per unit time was constant over the period of exposure. This is probably not the case. In reality, the amount of chemical or radiation that reached the cell should be greatest when the dose was initially instilled on the surface. In addition, the concentration of metabolites might change with time. The form of the model would remain the same if this were taken into consideration but the dose-rate would become an explicit function of time.

Future Research

Although there are uncertainties concerning the validity of the model in different situations, the model has been shown to be a good predictor of the experimental results presented here. Additional research in the following areas would clarify the uncertainties and strengthen the evidential base of the assumptions of the present model, consequently strengthening its precision and accuracy when it is applied more broadly.

[1] At this time it is not possible to discern where promotion ends and progression begins, or if the two stages

overlap. As a result, the stages have been collapsed together in this model. New information about the two stages, with an explicit modeling of tumor growth kinetics, could affect the predictions of the model.

[2] The initial state vector, the number of cells in states 0, 1, and 2 prior to any chemical or radiation insult, used in this study has been taken from in vitro studies. The values, if an initial state even exists in vivo, may be different and consequently lead to a different predicted risk of cancer.

[3] The results of this study have been obtained assuming that the cellular concentration of BaP has reached equilibrium. This suggests that there may be a problem if the ratio of BaP to its metabolites changes rapidly. It is important to know what the active metabolite is and its ratio to BaP in different cell populations and at different times following the onset of exposure.

[4] In order to simplify the model it was assumed that the dose to the organ is considered to be a constant fraction of the amount applied directly to the surface. In reality this is not the case, since there will be homogeneity of dose within cellular subpopulations. Doses to the epithelial cells will have to be estimated at some time to fully incorporate the toxicological data.

[5] The mechanism of contact inhibition removal is not well understood. Additional research would add insight into its role in the promotional stages of cell transformation.

The results presented in this study demonstrate that this model could prove useful in predicting the risks of chemical and radiation exposure *in vivo*. Applying this model, instead of the additive model advocated by the EPA, at Superfund sites or in polluted indoor air environments could provide a more accurate picture of the probability of developing cancer and may have a significant affect on clean-up or so called safe-levels presently advocated. In the future, an effort should be made to test this model with more complex combinations of chemicals and chemicals and/or radiation.

References

Armitage, P. and R. Doll. 1954. The Age Distribution of Cancer and A Multi-stage Theory of Carcinogenesis. Br. J. Cancer. 8(1):1-12.

Bivan, W.S., G.A. Olsen, and K.A. Murray. 1987. In Laboratory Hamsters. Van Hoosier, Jr., G.L. and C.W. McPherson, Eds. New York: Academic Press, Inc.

Chouroulinkov, I., A. Gentil, B. Tierney, P.L. Grover, and P. Sims. 1979. Biological Activities of Dihydrodiols Derived from Two Polycyclic Hydrocarbons in Rodent Test Systems. Br. J. Cancer. 39:376-382.

Cothern, C.R. and J.E. Smith Jr., Eds. 1987. Environmental Radon. Chapter 6:Dosimetry. Environmental Science Research, Vol. 35. New York: Plenum Press.

Crawford-Brown, D.J. and W. Hofmann. 1990a. A Generalized State-Vector Model for Radiation Induced Cellular Transformation. Int. J. Radiat. Biol. 57(2):407-423

Crawford-Brown, D.J. and W. Hofmann. (in press 1990b). A Mathematical Theory of the Influence of Dose-Rate on Cellular Transformation In-Vivo, to appear in Mathematical Biosciences, 1990.

DiPaolo, J.A., P. Donovan, and R. Nelson. 1969. Quantitative Studies of In Vitro Transformation be Chemical Carcinogens. J. Nat. Cancer Inst. 42:867-876.

Eastman, A., B.T. Mossman and E. Bresnick. 1981. Formation and Removal of Bap Adducts of DNA in Hamster Tracheal Epithelial Cells. Cancer Research. 41:2605-2610.

Farber, E. 1981. Chemical Carcinogenesis. New England J of Med. 305(23):1379-1389.

Gelboin, H., E. Huberman and L. Sachs. 1969. Enzymatic Hydroxylation of Benzopyrene and its Relationship to Cytotoxicity. Proc. Natl. Acad. Sci. 64:1188-1194

Grimmer, G., H. Brune, G. Dettbarn, K. Najack, U. Mohr and R. Wenzel-Hartung. 1988. Contribution of Polycyclic Aromatic Compounds to the Carcinogenicity of Sidestream Smoke of Cigarettes Evaluated by Implantation into the Lungs of Rats. Cancer Letters. 43:173-177.

Hall, E.J. and T.K. Hei. 1985. Oncogenic Transformation with Radiation and Chemicals. Int. J. Radiat. Biol. 48(1):1-18.

Hieber, L., C.K. Hill, M.M. Elkind. 1987. Absence of dose-rate effect in the transformation of C3H/10T1/2 Cells by Alpha particles. International Journal of Radiation Biology. 52:859-869.

Kells, L.M., 1960. Elementary Differential Equations. New York: McGraw-Hill.

Kennedy, A.R., A. Desrosiers, M. Terzaghi, and J.B. Little. 1978. Morphometric and Histological Analysis of the Lungs of Syrian Golden Hamsters. J. Anat. 125(3):527-553.

Little, J.B., R.B. McGandy and A.R. Kennedy. 1978. Interactions between Polonium-210 alpha-radiation, Benzo(a)pyrene, and 0.9% NaCl Solution Instillations in the Induction of Experimental Lung Cancer. Cancer Research. 38:1929-1935.

Little J. 1990. Personal Communication via telephone between J. Little, Department of Radiation Biology, Harvard School of Public Health and D. Martin. Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill. February 22, 1990.

Lloyd, E.L., M.A. Gemmill, C.B. Henning, D.S. Gemmell, and B.J. Zabransky. 1979. Cell Survival Following Multiple-Track Alpha Particle Irradiation. Int. J. Radiat. Biol. 35(1):23-31.

McGandy, R.B., A.R. Kennedy, M. Terzgahi, and J.B. Little. 1974. Experimental Respiratory Carcinogenesis: Interaction between Alpha Radiation and Benzo(a)pyrene in the Hamster. In Experimental Lung Cancer: Carcinogenesis and Bioassays. eds. A. Karbe and J.F. Park. International Symposium, Batelle Seattle Research Center. Seattle WA, USA, June 23-26. New York: Springer-Verlag. pp. 485-491.

Moolgavkar, S. 1978. The Multistage Theory of Carcinogenesis and the Age Distribution of Cancer in Man. J. Natl. Cancer. Inst. 61(1):49-52.

Moolgavkar, S. and A.G. Knudson, Jr. 1980. Mutation and Cancer: A Model for Human Carcinogenesis. J. Natl. Cancer Inst. 66(6):1037-1052.

Moshandreas, J.D., J. Zabransky, and D.J. Peltas. 1981. Comparison of Indoor and Outdoor Air Quality. Menlo Park, CA: Electric Power Research Institute. (Report EA-1733) (as reported in Samet et al. 1987).

National Academy of Sciences (NAS). 1988. Health Risks from Radon and other Internally Deposited Alpha-Emitters (BIER IV). National Research Council (NRC), Committee on the Biological Effects of Ionizing Radiation (BEIR). Washington, D.C.: National Academy Press.

National Council on Radiation Protection and Measurements (NRCP). 1989. Comparative Carcinogenicity of Ionizing Radiation and Chemicals: Recommendations of the National Council on Radiation Protection and Measurements. Report #96. Bethesda, MD: The Council.

National Research Council (NRC). 1986. Environmental Tobacco Smoke: Measuring Exposures and Assessing Health Effects. Committee on Passive Smoking. Board on Environmental Studies and Toxicology. Washington, D.C.: National Academy Press.

Nordling, C.O. 1953. A New Theory on the Cancer-inducing Mechanism. Br. J. Cancer. 7:68-72.

Pitot, H. 1986. Principles of Oncology. 3d edition. New York: Dekker.

Repace, J.L., and A.H. Lowrey. 1980. Indoor Air Pollution, Tobacco Smoke, and Public Health. Science. 208:464-472.

Samet, J. M., M.C. Marbury, and J.D. Spengler. 1987. Health Effects and Sources of Indoor Air Pollution, Part I. American Review of Respiratory Disease. 136:1486-1508.

Scott, B.R. and E.J. Ainsworth. 1980. State-Vector Model for Life Shortening in Mice After Brief Exposures to Low Doses of Ionizing Radiation. Mathematical Biosciences. 49:185-205.

Shuman, J.P. 1989. A Theoretical Study of the Probability of Developing Transformed Cells Due to In-Vivo Irradiation by "Hot Particles" Using a Generalized Five State-Vector Model. Masters Thesis. Department of Environmental Science and Engineering, School of Public Health. University of North Carolina at Chapel Hill.

Spengler, J.D., D.W. Dockery, W.A. Turner, J.M. Wolfson, and B.G. Ferris. 1981. Long-term Measurements of Respirable Sulfates and Particles Inside and Outside Homes. Atmos. ENVIR. 15:23-30. (as reported in Samet et al. 1987).

U.S. Environmental Protection Agency (USEPA). 1987. Superfund: Looking Back, Looking Ahead. Office of Public Affairs (A-107). Washington, D.C.: USEPA. OPA-87-007. December.

U.S. Environmental Protection Agency (USEPA). 1986. Superfund Public Health Evaluation Manual. Office of Emergency and Remedial Response. Washington, D.C.: USEPA. EPA 540/1-86/060.

Bibliography

Bender, M.A., H.G. Griggs, and J.S. Bedford. 1974. Mechanisms of Chromosomal Aberration Production: III. Chemicals and Ionizing Radiation. Mutation Research. 23:197-212.

Bresnick, E., T.F. McDonald, H. Yagi, D.M. Jerina, W. Levin, A.W. Wood, and A.H. Conney. 1977. Epidermal Hyperplasia after Topical Application of Benzo(a)pyrene Diol Epoxides, and Other Metabolites. Cancer Research. 37: 984-990.

Chambers, R.W. 1985. Chemical Carcinogenesis: A Biochemical Overview. Clinical Biochemistry. 18:158-168.

Chameaud, J., R. Perraud, J. LaFuma, R. Masse and J. Pradel. Lesions and Lung Cancers Induced in Rats by Inhaled Radon 222 at Various Equilibriums with Radon Daughters. In Experimental Lung Cancer: Carcinogenesis and Bioassays. eds. A. Karbe and J.F. Park. International Symposium, Batelle Seattle Research Center. Seattle WA, USA, June 23-26. New York: Springer-Verlag. pp. 411-421.

Chan, G.L. and J.B. Little. 1986. Neoplastic Transformation In Vitro. In Radiation Carcinogenesis. eds. A.C. Upton, R.E. Albert, F.J. Burns, and R.E. Shore. New York, New York: Elsevier Science Publishing Co. pp.107-136.

Chouroulinkov, I., A. Gentil, P.L. Grover, and P. Sims. 1976. Tumour-Intiating Activities on Mouse Skin of Dihydrodiols Derived from Benzo(a)pyrene. Br. J. Cancer. 34:523-532.

Crawford-Brown, D.J. 1988. The Microscopic Structure of Radiation Interactions in Cells. Sci. Prog. Oxf. 72:53-74.

DiPaolo, J.A. 1971. X-Irradiation Enhancement of Transformation by Benzo(a)pyrene in Hamster Embryo Cells. Proc. Nat. Acad. Sci. 68(8):1734-1737.

DiPaolo, J.A. 1976. In Vitro Transformation: Interactions of Chemical Carcinogens and Radiation. In Biology of Radiation Carcinogenesis. eds. J.M. Yuhas, R.W. Tennant and J.D. Regan. New York: Raven Press. pp. 335-342.

Elkind, M.M. 1984. Failla Memorial Lecture: Repair Processes in Radiation Biology. Radiation Research 100:425-449.

Farber, E. 1987. Possible Etiologic Mechanisms in Chemical Carcinogenesis. Environmental Health Perspectives. 75:65-70.

Farrell, R.L. and G.W. Davis. 1974. Effect of Particulate Benzo(a)pyrene Carrier on Carcinogenesis in the Respiratory Tract of Hamsters. In Experimental Lung Cancer: Carcinogenesis and Bioassays. eds. A. Karbe and J.F. Park. International Symposium, Batelle Seattle Research Center. Seattle WA, USA, June 23-26. New York: Springer-Verlag. pp. 186-198.

Hoffmann, D. and E.L. Wynder. 1986. Chemical Constituents and Bioavailability of Tobacco Smoke. International Agency for Research of Cancer Sci. Publication #74. Lyon, France: IARC.

Hoffmann, D., E.L. Wynder, S.S. Hecht, K.D. Brunnemann, E.J. LaVoie, and N.J. Haley. 1987. Chemical Carcinogens in Tobacco. In Cancer Risks: Strategies for Elimination. ed. P. Bannasch. New York: Springer-Verlag. pp. 101-113.

Hsu,W-T., E.J.S. Lin, R.G. Harvey, and S.B. Weiss. 1977. Mechanism of phage 0X174 DNA Inactivation by Benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide. Proc. Natl. Acad. Sci. 74(8):3335-3339.

Huberman, E., L. Sachs, S.K. Yang, and H.V. Gelboin. 1976. Identification of Mutagenic Metabolites of Benzo(a)pyrene in Mammalian Cells. Proc. Natl. Acad. Aci. 73(2):607-611.

International Agency for Research on Cancer (IARC). 1973. Benzo(a)pyrene. IARC Monographs on the Evaluation of Carcinogenic Risk of the Chemical to Man: Certain polycyclic aromatic hydrocarbons and heterocyclic compounds. Vol. 3. Lyon, France: IARC. pp.91-136.

International Agency for Research on Cancer (IARC). 1983. Benzo(a)pyrene. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental, and Experimental Data. Volume 32. 211-224.

Jones, C.A., R.M. Santella, E. Huberman, J.K. Selkirk and D. Grunberger. 1983. Cell Specific Activation of Benzo(a)pyrene by Fibroblasts and Hepatocytes. Carcinogenesis. 4(11):1351-1357.

Kennedy, A.R. and J.B. Little. Cellular Localization of Intratracheally Administered 210Po in the Hamster Lung Using Autoradiography of Thin Sections from Plastic Embedded Tissue. In Experimental Lung Cancer: Carcinogenesis and Bioassays. eds. A. Karbe and J.F. Park. International Symposium, Batelle Seattle Research Center. Seattle WA, USA, June 23-26. New York: Springer-Verlag. pp. 475-484.

Lane, B.P. and S.L. Miller. 1974. Dose Dependence of Carcinogen-Induced Changes in Tracheal Epithelium in Organ Culture. In Experimental Lung Cancer: Carcinogenesis and Bioassays. eds. A. Karbe and J.F. Park. International Symposium, Batelle Seattle Research Center. Seattle WA, USA, June 23-26. New York: Springer-Verlag. pp. 507-513.

Levin, A., A.W. Wood, H. Yagi, D.M. Jerina, and A.H. Conney. 1976. (+/-)-trans-7,8-Dihydroxy-7,8-dihydrobenzo(a)pyrene: A Potent Skin Carcinogen When Applied Topically to Mice. Proc. Natl. Acad. Sci. 73(11):3867-3871.

Lisco, H., A. R. Kennedy, and J.B. Little. 1974. Histologic Observations in Hamsters Following Administration of Polonium-210. In Experimental Lung Cancer: Carcinogenesis and Bioassays. eds. A. Karbe and J.F. Park. International Symposium, Batelle Seattle Research Center. Seattle WA, USA, June 23-26. New York: Springer-Verlag. pp. 468-474.

Little, J.B. and W.F. O'Toole. 1974. Respiratory Tract Tumors in Hamsters Induced by Benzo(a)pyrene and 210 Po alpha-radiation. Cancer Research. 34:3026-3039.

Lockhart, M.L. and B. H. Rosenberg. 1983. Inhibition of DNA Synthesis, Independent of DNA Adduct Formation, by Benzo(a)pyrene Diol Epoxide in Mammalian Cells. Carcinogenesis. 4(2):125-130.

Marquardt, H., P.L. Grover, and P. Sims. 1976. In Vitro Malignant Transformation of Mouse Fibroblasts by Non-K-Region Dihydrodiols Derived for 7-Methylbenz(A)anthracene, 7,12-Dimethylbenz(a)anthracene, and Benzo(a)pyrene. Cancer Research. 36: 2059-2064.

McDowell, E.M., and B.J. Trump. 1983. Histogenesis of Preneoplastic and Neoplastic Lesions in Tracheobronchial Epithelium. Surv. Synth. Path. Res. 2:235-279.

Metivier, H., J. Wahrendorf, and R. Masse. 1984. Multiplicative Effect of Inhaled Plutonium Oxide and Benzo(a)pyrene on Lung Carcinogenesis in Rats. Br. J. Cancer. 50:215-221.

Newbold, R.F. and P. Brookes. 1976. Exceptional Mutagenicity of a Benzo(a)pyrene Diol Epoxide in Cultured Mammalian Cells. Nature. 261:52-54.

Saracci, R. 1987. The Interactions of Tobacco Smoking and Other Agents in Cancer Etiology. The Johns Hopkins School of Hygiene and Public Health. Epidemiologic Reviews. 6:175-193.

Slaga, T.J., A. Viaje, D.L. Berry, W. Bracken, S.G. Buty, and J.D. Scribner. 1976. Skin Tumor Initiating Ability of Benzo(a)pyrene 4,5-, 7,8- and 7,8-Diol-9,10-Epoxides and 7,8-Diol. Cancer Letters. 2:115-122.

Slaga, T.J., S.M. Fischer, A. Viaje, D.L. Berry, W.M. Bracken, S. LeClerc, and D.R. Miller. 1978. Inhibition of Tumor Promotion by Antiinflammatory Agents: An Approach to the Biochemical Mechanism of Promotion. In Carcinogenesis. Vol. 2. Mechanisms of Promotion and Cocarcinogenesis. eds. T.J. Slaga, A. Sivak, and R.K. Boutwell. New York: Raven Press. pp. 173-195.

Sims, P., P.L. Grover, A. Swaisland, K. Turner, and A. Hewer. Sims, P., P.L. Grover, A. Swaisland, K. Pal. and A. Hewer. 1974. Metabolic Activation of Benzo(a)pyrene proceeds by a Diol-Epoxide. Nature. 252:326-327.

Terzaghi, M. and J.B. Little. 1974. Interactions between Radiation and Benzo(a)pyrene in an in vitro Model for Malignant Transformation. In Experimental Lung Cancer: Carcinogenesis and Bioassays. eds. A. Karbe and J.F. Park. International Symposium, Batelle Seattle Research Center. Seattle WA, USA, June 23-26. New York: Springer-Verlag. pp.497-505.

Thyssen, J., J. Althoff, G. Kimmerle, and U. Mohr. 1981. Inhalation Studies with Benzo(a)pyrene in Syrian Golden hamsters. JNCI. 66(3):575-577.

Wislocki, P.G., A.W. Wood, R.L Chang, W. Levin, H. Yagi, O. Hernandez, P.M. Dansette, D.M. Jefina, and A.H. Conney. Mutagenicity and Cytotoxicity of Benzo(a)pyrene Arene Oxides, Phenols, Quinones, and Dihydrodiols in Bacterial and Mammalian Cells. Cancer Research. 36: 3350-3357.

Wood, A.W., P.G. Wislocki, R.L. Chang, W. Levin, A.Y.H.Lu, H. Yagi, O. Hernandez, D.M. Jerina, and A.H. Conney. 1976. Mutagenicity and Cytotoxicity of Benzo(a)pyrene Benzo-Ring Epoxides. Cancer Research. 36:3358-3366.

Zeller, W.J. and M.R. Berger. 1988. Combination Effects of Tobacco Constituents in Experimental Carcinogenesis. In Combination Effects in Chemical Carcinogenesis. ed. D. Schmahl. Weinheim, W. Germany: VCH. pp. 175-191.

1000 EDITION

ANNIVERSARY BOND

BY

FOX PIKER