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Title: ANALYSIS OF DICHOTOMOUS RESPONSE MODELS FOR LOW-DOSE CARCINOGENIC RISK ESTIMATION

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Several dichotomous-response models of carcinogenesis are discussed and their implications for low-dose risk estimation are examined. In addition, a pharmacokinetic model incorporating nonlinear kinetics is studied and the results are investigated with respect to multiple-pollutant exposures. None of these models appears to suggest the existence of "thresholds".

The analysis of both linear and nonlinear models indicates that if background concentrations are present, then under almost all models discussed, the marginal response associated with the new carcinogen increases more linearly with dose.

The results of the study are then examined with respect to dispersal of carcinogenic pollutants as a means of reducing environmental hazards to a given population. It was then concluded that dispersal may not reduce the overall risk if linear or near-linear dose-response relationships are assumed.

A conceptual experimental design is also presented to investigate a general relationship between dispersal and total population incidence.

Analysis of Dichotomous response Models for Low-Dose Carcinogenic Risk Estimation

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CHAPTER I

INTRODUCTION

Humans are exposed to environmental and occupational carcinogens of varying degrees of tumorogenicity. Some occur naturally, some are highly potent and can induce neoplastic growth at fractions of a part per billion, while others are highly persistent and result in long-term exposures.

Epidemeologic studies of humans exposed to already present carcinogenic agents are integral elements of risk estimation procedures. Unfortunately, neoplastic developments are associated with long periods of time. It may take months, years, or perhaps decades from the first exposure before the tumor is clinically detected. Furthermore, epidemeologic approaches cannot predict risks associated with potential carcinogenic chemicals yet to be introduced to the environment.

The traditional approach for assessment of risk to a population has been to expose a relatively small group of laboratory animals (rodents) to high doses of a given carcinogen and extrapolate the results to low concentrations by means of the present mathematical models of chemical carcinogenesis.

The high concentration levels applied to laboratory animals seldom occur in environmentally stable conditions. However, in order

to obtain statistically significant results within reasonable confidence intervals, one could either attempt to monitor large groups of animals for tumor incidence due to lose-dose exposures (e.g. mega mouse study), or apply high doses of the pollutant to a small group. The former has been proven an unmanageable and impractical situation to be considered for thousands of potential carcinogenic substances. The latter is associated with the problem that would arise from low-dose extrapolations - namely, one would need to use high-dose data to predict a dose at which cancer incidence would exceed the background occurrence by maximum of a prespecified number, such as 10^{-6}.

Dichotomous response models are applied to high-dose data to estimate increases in risk (or cancer incidence) with incremental increases in dose. Three dichotomous response models (one-hit, multi-hit, and multistage models) of carcinogenesis are investigated. In addition, the log-probit model (following a different rationale) is included in the discussions. A pharmacokinetic example incorporating Michaelis-Menten kinetics is also discussed with regard to low dose implications and presence of multiple pollutants.

One may note that conditions created in chemical carcinogenesis experiments generally do not simulate those encountered by humans. Clean laboratory animals of known strain and genetic characteristics are exposed to high levels of a single pollutant. On the contrary, humans are exposed to chronic levels of carcinogens of various structure, reactivity and potency. Experimental designs simulating human

exposures are therefore required to provide more applicable results in chemical carcinogenesis studies.

This study attempts to clarify the assumptions of the existing dichotomous response models with respect to multiple carcinogens and background concentrations. In addition, a conceptual experiment is proposed to examine the effects of pollutant dispersal on a given uniform population.

CHAPTER II

DICHOTOMOUS RESPONSE MODELS FOR CARCINOGENESIS

A number of statistical models have been developed to describe carcinogenic processes leading to induction and detection of tumors. At the present time, none of these models have gained general recognition from the scientific community, principally because the experiments performed on laboratory animals do not yield statistically significant results to assist extrapolations of incidence rates to low doses of carcinogens. For example, if 1000 animals were used for assay at a single dose and no tumors were detected, then there would exist a 95% confidence level for the true incidence to be less than 0.5%. This incidence rate, however, would represent approximately one million people in the United States , which is clearly an unacceptable level of risk.

Detection of more realistic incidence rates of 10^{-8} - 10^{-5} would require millions of rodents for obtaining statistically meaningful results, considering the pool of present carcinogens. Experiments of this nature are unlikely to be successful or economically possible.

Risk at low doses is, therefore, estimated by exposing a small group of rodents to high doses of carcinogenic agents and extrapolating the results for low values of dose by means of existing mathematical models.

Problems also arise from extrapolating results of animal experiments to humans. On the basis of epidemeologic studies, certain chemicals (for example, β -naphtylamine) which are well known as human carcinogens, do not appear to be carcinogenic in laboratory animals (IARC, 1974). Generally, however, there exists a reasonable correlation between results of animal carcinogenic experiments and those of human tumor incidence rates.

A cautious review of the mathematical models of carcinogensis, therefore, requires consideration of the problems associated with risk evaluation at low doses, animal-to-man extrapolations, synergistic (or antagonistic) effects of one pollutant in the presence of the carcinogenic agents, and metabolic xanabilities within a population.

It is the purpose of this chapter to elucidate the assumptions and investigate the implications regarding dichotomous models of carcinogenesis.

2.1 One Hit Dose-response Model of Carcinogenesis

The one-hit, or linear model of carcinogenesis, is based on the assumptions that the growth of a cancer tumor is initiated by a one-step transition induced by a carcinogenic agent and occuring in a single cell; and that the time-to-development of the transformed cell is independent of initial dose.

Stochastic processes by which the transitions occur describe the probabilities of tumor induction as functions of dose or exposure. The actual carcinogenic process, however, involves biochemical and

physiological mechanisms and reactions, a process which involves repair and destruction as well as neoplastic growth.

Assumptions regarding the one-hit model are:

1. A cancer tumor originates from a single cell
2. A single dose of the specific carcinogen can induce the growth of a cancer tumor (Crump et al., 1976), whose development period is independent of dose.

In effect, the model assumes that the growth of a tumor is initiated when one molecule of carcinogen "hits" one critical site within a single cell. The process by which these hits occur is assumed to be a stochastic process following a Poisson approximation to the binomial distribution.

Recall that the probability distribution of the Poisson random variable X, representing the number of successes occuring in a given time interval is given by:

$$P(x, \mu) = \frac{e^{-\mu x}}{x!}, \quad = 0, 1, 2, \ldots \qquad \text{eq. 2-1}$$

where Γ is the average number of successes occurring in the given time interval.

For $\mu = \lambda D$, where λD is the average number of hits at dose D, the probability of exactly k hits is

$$P(X = k; \lambda D) = \frac{e^{-\lambda D}(\lambda D)^k}{k!}, \quad k = 0, 1, 2, \ldots \qquad \text{eq. 2-2}$$

where λ is an unknown constant representing the slope of the dose-response curve at zero dose (or the risk of tumor induction per unit of dose D).

Assumption 2 states that only one "hit" is sufficient to induce the growth of a tumor, therefore for $k \geq 1$ (one hit or more),

$$P(D) = P(k \geq 1) = 1 - P(k < 1) = 1 - P(k=0) = 1 - e^{-\lambda D} \qquad \text{eq. 2-3}$$

Expanding the exponential term in equation 2.3,

$$P(k \geq 1) = 1-e^{-\lambda D}=1 - [1-\lambda D + \frac{(\lambda D)^2}{2!} - \frac{(\lambda D)^3}{3!} + \ldots] \qquad \text{eq. 2-4}$$

for small λD, i.e. for small doses of carcinogen, the higher order terms in equation 2-4 become negligible, thus

$$P(D) \simeq \lambda D \qquad \text{eq. 2-5}$$

The above result indicate that for low values of dose, the slope of the dose-response curve is constant and no threshold occurs, that is, the response is linearly proportional to dose. In effect, at low dose regions, the model assumes that the number of hits is proportional to the amount of carcinogen and the rate of tumor production is proportional to the number of hits (see Figure 2.1).

Note that the abscissa of the curve in Figure 2.1 is the effective dose, D, which is the sum of the administered dose d, and a background dose δ. The probability of response due to administered dose is illustrated in Figure 2.2.

At this point, several remarks must be made regarding the assumptions made in the derivation of the one-hit model.

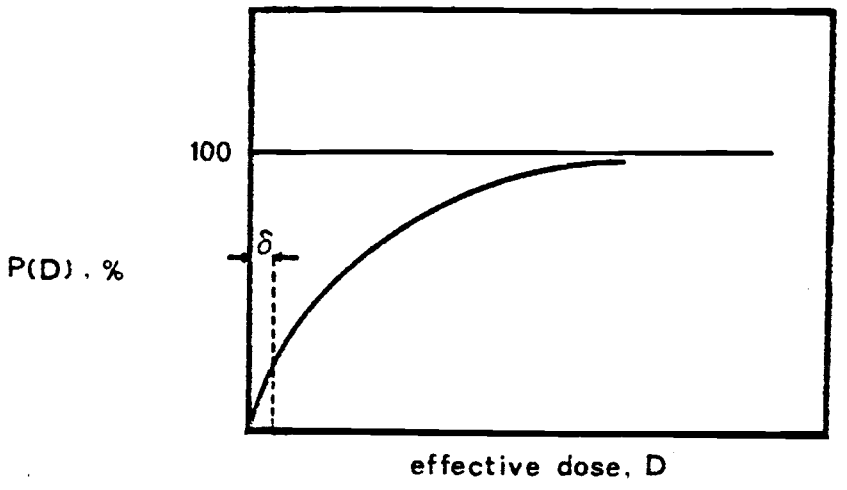

Figure 2.1. Probability of tumor as a function of effective dose, D, using one-hit model of carcinogenesis

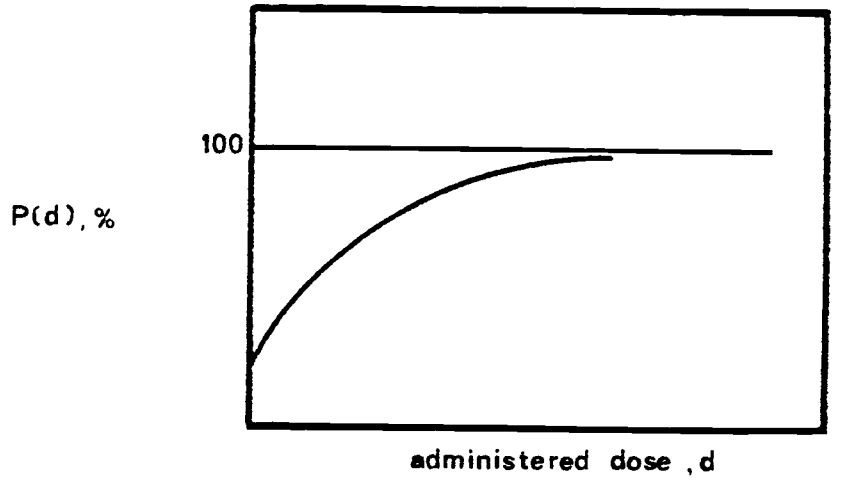

Figure 2.2. Probability of response (percent of population) as a function of administered dose, d, using one-hit (linear) model of carcinogenesis

Two observations support the one-cell origin of cancers (assumption 1). First, in women heterozygous for electrophoretic variants of X-linked glucose-6-phosphate dehydrogenase, neoplastic tissues are distinctly of one phenotype or the other, whereas the normal tissue consists of mixtures of cells representing either one of the two phenotypes (Crump et al., 1976).

The second evidence in support of assumption 1 comes from experimental studies of single transformed cells transplanted in animal tissues. These cells seem to be well able to give rise to neoplasms (Gartler, 1974).

The second assumption stating that induction time is independent of dose is not a strong assumption in the sense that very high doses could definitely result in decreased induction time. However, environmental doses of carcinogens are small enough to make this assumption appropriate for low dose carcinogenic risk assessment.

2.2 Multi-hit Model

The multi-hit model of carcinogenesis assumes that at least k number of transitions or "hits" are required in order to initiate tumor growth. The one-hit model can be considered a special case of this model for k = 1. The assumption of single-cell origin made in discussion of the one-hit model also applies to the multi-hit model, however the requirement of at least k hits would result in modification of equation 2-1. In essence, the multi-hit model can be formulated as follows (Dänzer, 1934):

The Dänzer Formulation

1. The cell has m critical sites
2. The cell will be transformed to a cancer cell if at least k of these critical sites are "hit", each by ℓ or more quanta of carcinogenic agents.
3. The probability p_i that a given quantum (effective unit) of dose will hit the ith critical site is constant. The probability p_0 that a quantum of dose will miss the ith critical site is also constant (the Bernoulli Postulate).
4. If nD is the total effective dose (where n = number of effective dose units and D = dose), then the probability that there would be exactly k hits on the ith critical site is given by binomial distribution:

$$P(k) = \binom{nD}{k} p_i^{\,k} q_i^{nD-k}$$

$$= \frac{nD\ (nD-1)\ \ldots\ (nD-k+1)}{k!}\, p_i^{\,k}\, q_i^{(nD-k)} \qquad \text{eq. 2-6}$$

where $k = 0, 1, 2, \ldots, nD$, $i = 0, 1, 2, \ldots m$

and $q_i = 1 - p_i$

5. If nDp_i is of the order of 1, then the following estimate, known as the Poisson Theorem, can be used (Papoulis, 1965).

$$\frac{(nD)!}{k!\ (nD-k)!}\ p_i^k\ q_i^{nD-k} \simeq e^{-nD\,p_i}\ \frac{(nD\ p_i)^k}{k!} \qquad \text{eq. 2-7}$$

for k of the order of nDp_i. This result can be stated as a limit if

$$nD \longrightarrow \infty \quad p_i \longrightarrow 0 \qquad nDp_i \longrightarrow \lambda D$$

Then

$$\frac{(nD)!}{k!\ (nD-k)!}\ p_i^k\ q_i^{nD-k} \longrightarrow e^{-\lambda D}\ \frac{(\lambda D)^k}{k!} \qquad \text{Eq. 2-8}$$

Equation 2-8 can be justified by using quantitative reasoning. Since k is of the order of np_iD and $p_i << 1$, it can be concluded that k nD. Therefore, in the numerator of 2-6 all factors can be approximated by nD. Thus

$$nD\ (nD - 1)\ldots.\ (nD - k+1) \simeq nD\ .\ nD\ldots\ldots nD = (nD)^k \qquad \text{Eq. 2-9}$$

Since $p_i << 1$, and $kp_i << 1$

$$q_i = 1 - p_i \simeq e^{-p_i} \qquad \text{Eq. 2-10}$$

$$q^{(nD-k)} \simeq e^{-(nD-k)\ p_i} \simeq e^{-np_iD} = e^{-\lambda_i D} \qquad \text{Eq. 2-11}$$

Substituting the above approximations in equation 2-6, the result in 2-8 can be obtained.

6. $\lambda_1 = \lambda_2 = \ldots\ \lambda_i = \ldots. = \lambda_m$ (similar site postulate)

   The requirement of <u>at least</u> k "hits" will result in the following probability distribution:

$$P(D) = P\ (\alpha \geq k) = 1\text{-}P\ (a < k)$$

$$= \sum_{i=0}^{k-1} \frac{(\lambda D)^i\ \bar{e}^{\lambda D}}{i!} \qquad \text{Eq. 2-12}$$

Equation 2-12 is an expansion of the one-hit model and therefore, could be a better description of dose-response relationships.

For k = 1, equation 2-12 will become

$$P(D) = 1 - e^{-\lambda D} \qquad \text{Eq. 2-13}$$

where $\lambda = \lambda_1 = \lambda_2 = ... = \lambda_m$. The above result is consistent with the one-hit model of carcinogenesis. Allowing k to be any real number, equation 2-12 can be modified to (see Appendix A):

$$P(D) = 1 - \frac{1}{\Gamma(k)} \int_0^{\lambda D} e^{-U} U^{(k-1)} \, dx \qquad \text{Eq. 2-14}$$

where $\Gamma(k) = (k-1)\,(k-2)\ldots\Gamma(1)$ and $\Gamma(1) = \int_0^{\infty} e^{-U} \, dx = 1.$

Expanding equation 2-12:

$$P(D) = 1 - \sum_{i=0}^{k-1} \frac{(\lambda D)^i \, e^{-\lambda D}}{i!} = 1 - e^{-\lambda D} \left[\sum_{i=0}^{k-1} \frac{(\lambda D)^i}{i!} \right]$$

$$= 1 - e^{-\lambda D} \left\{ \sum_{i=0}^{\infty} \frac{(\lambda D)^i}{i!} - \sum_{i=k}^{\infty} \frac{(\lambda D)^i}{i!} \right\}$$

For small λD

$$P(D) = 1 - 1 + \sum_{i=k}^{\infty} \frac{(\lambda D)^i}{i!} = \frac{(\lambda D)^k}{k!} + \frac{(\lambda D)^{k+1}}{(k+1)!} + \ldots \qquad \text{Eq. 2-15}$$

For small values of λD, the above equation can be approximated as follows:

$$P(D) \simeq \frac{(\lambda D)^k}{k!} \qquad \text{Eq. 2-16}$$

Equation 2-15 can also be written as:

$$P(D) \simeq 1 - \sum_{i=0}^{k-1} \frac{(\lambda D)^i}{i!} [1-\lambda D + \frac{(\lambda D)^2}{2!} - \frac{(\lambda D)^3}{3!} + \ldots] \qquad \text{Eq. 2-17}$$

The term in the brackets is the series expansion of $e^{-\lambda D}$. For $k = 1$, and small values of λD equation 2-17 can be approximated as

$$P(D) \simeq \lambda D \qquad \text{Eq. 2-18}$$

which represents a linear response at low doses.

Going back to equation 2-16, for small values of λD,

$$P(D) \simeq \frac{\lambda^k D^k}{k!} = \nu_k D^k \qquad \text{Eq. 2-19}$$

where $\nu_k = \frac{\lambda^k}{k!}$. Equation 2-19 implies that response increases as a power of dose. The plots in Figure 2.3 represent the dose-response relationships for $k = 1$ and $k > 1$.

2.3 Multistage Model

The criticism that the multi-hit model has encountered originates from the fact that cell's time-to-response has not been included in the derivations of the model. Crump et al. (1976) stated that if a cell has a time-to-respose, where response could mean death due to cancer, then this time could be written as

$$t_r = t_a + t_g$$

where t_r = time-to-response, t_a = time-to-alteration

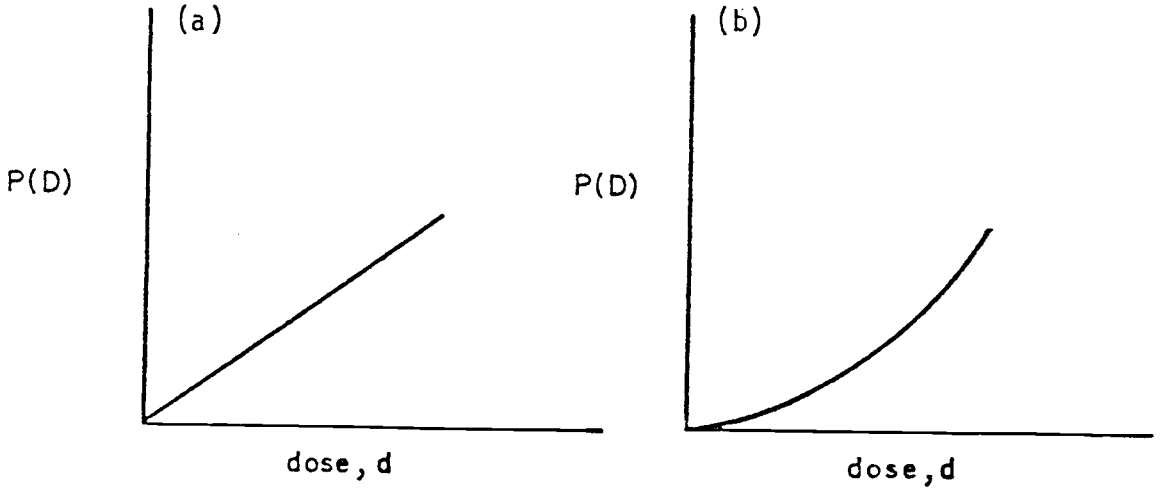

Figure 2.3. Dose-response relationships according to multi-hit model of carcinogenesis (a), k=1; (b), k > 1.

of a normal cell to a malignant cell, and t_g = time from complete alteration to death. t_a would presumably be dependent on dose D, whereas the effect of D on t_g is assumed to be negligible. The response can then be written as

$$I_s(t,D) = \int_0^t I_a\ (t-U,D)\ f(U)\ dU \qquad \text{Eq. 2-20}$$

where $I_a(t,D)$ is the incidence rate of alteration of one cell from normal to malignant, f(t) is the density function for cancer growth time, and $I_s(t,D)$ is the incidence rate of cancer response for a single cell.

If a tissue is composed of N cells, the incidence rate of the whole tissue responding to dose D, can be written as

$$I_w\ (t,D) = NI_s\ (t,D) \qquad \text{Eq. 2-21}$$

since the time-to-response for the whole tissue would be the smallest of the response times for N cells.

Armitage and Doll (1961) showed that the time rate of occurrence of the ith event is $\alpha_i + \beta_i D$ (where α_i = spontaneous background occurrence or response, β_i = porportionality constant for the carcinogenic process induced by dose at each stage i, i = 1,...., k, and $\alpha_i,\ \beta_i \simeq 0$).

$I_a\ (t,D)$, rate of occurance of k completed stages would then be given as

$$I_a(t,D) = kt^{k-1}\left\{\prod_{i=1}^{k}(\alpha_i + \beta_i D) = kt^{k-1}\ Q_k(D)\right\} \qquad \text{Eq. 2-22}$$

where $Q_k(D)$ is a kth degree polynomial in D. Equations 2-20, 2-21, and 2-22 result in the following relation:

$$I_w(t,D) = [Nk \int_0^t f(t-y)\, y^{k-1}\, dy]\, Q_k(D) = \xi_k(t)\, Q_k(D) \qquad \text{Eq. 2-23}$$

Taylor series expansion of $Q_k(D)$ would result in the following expression

$$P_w(t,D) = [Nk \int_0^t f(t-U)\, U^{k-1}\, dU]\, [a + bD + R(D)] \qquad \text{Eq. 2-24}$$

where a and b are constants and R(D) is negligible. For low values of dose D levels, therefore, the incident rate is given by:

$$I_w(t,D) = [Nk \int_0^t f(t-y)\, y^{k-1}\, dy]\, [a + bD] \qquad \text{Eq. 2-25}$$

which is linear in dose.

The significance of the above results was discussed by Crump et al. (1976). The multistage model of carcinogenesis developed by Armitage and Doll (1954) would result in a similar expression as one obtained in 2-24. The only difference between the multi-hit and multistage processes is that the k "hits" must occur in a particular time sequence in the multistage model. Their model can be expressed as

$$P(D,t) = 1-e^{-\tau(t)\cdot\Theta(D)}$$

where

$$\Theta(D) = \prod_{i=1}^{k} (\alpha_i + \beta_i D) \qquad \text{Eq. 2-26}$$

$\Theta(\tau)$ is a function of exposure time, but is independent of dose and D is the dose rate. This model is a more general form of multi-hit model represented by equation 2-12. The implication of cancer incidence rate increasing as a high power of time closely approximates incidence of cancer in humans.

Hoel (1976) discussed the multi-stage models in their low-dose regions and concluded that the extent of linearity would depend on the background level of dose. This brings into attention the assumption that the induced carcinogenic process due to administered dose d is merely in sequence with the spontaneous process due to background dose and not independent of it.

2.4 Log-Probit Model

The log probit model for carcinogenesis follows a different rationale. It assumes that each member of the population has its own threshold concentration above which, a tumor is produced by exposure to the chemical. The distribution of the log-dose thresholds is assumed to be Gaussian (normal). At low concentrations only a few thresholds are exceeded and thus the tumor incidence is low. At higher concentrations, additional thresholds are exceeded. At very high concentrations only a few thresholds are not exceeded. Thus the

dose-response curve displays a sigmoid shape with the most frequent threshold levels found at the inflection point of the curve (see Figure 2.4).

This model is mathematically expressed as

$$P(D) = \frac{1}{\sqrt{2\pi}(\log \sigma)} x \int_{-\infty}^{\log D} \exp\left[-\frac{1}{2}\left(\frac{x - \log D_g}{\log \sigma}\right)\right] dx \qquad \text{Eq. 2-27}$$

where σ is the geometric standard deviation and D_g is the geometric mean (Altshuler, 1981).

This model was adopted by Cornfield (1977) to describe the existence of a threshold or a safe dose for chemical carcinogens. However, thresholds, if they exist, vary over time within an individual due to such factors as age or concurrent diseases, as well as among individuals in a given population. This variability is extremely important from a regulatory point of view since all individuals must be protected at all times. Thus, even if log probit, model is used, it is inappropriate to suggest that with respect to an entire population a given carcinogen exhibits a threshold or zero-response concentration level. This model, by no means, postulates the existence of single population thresholds.

Although the log-probit model has been used in many experimental situations, the second problem associated with the use of this model

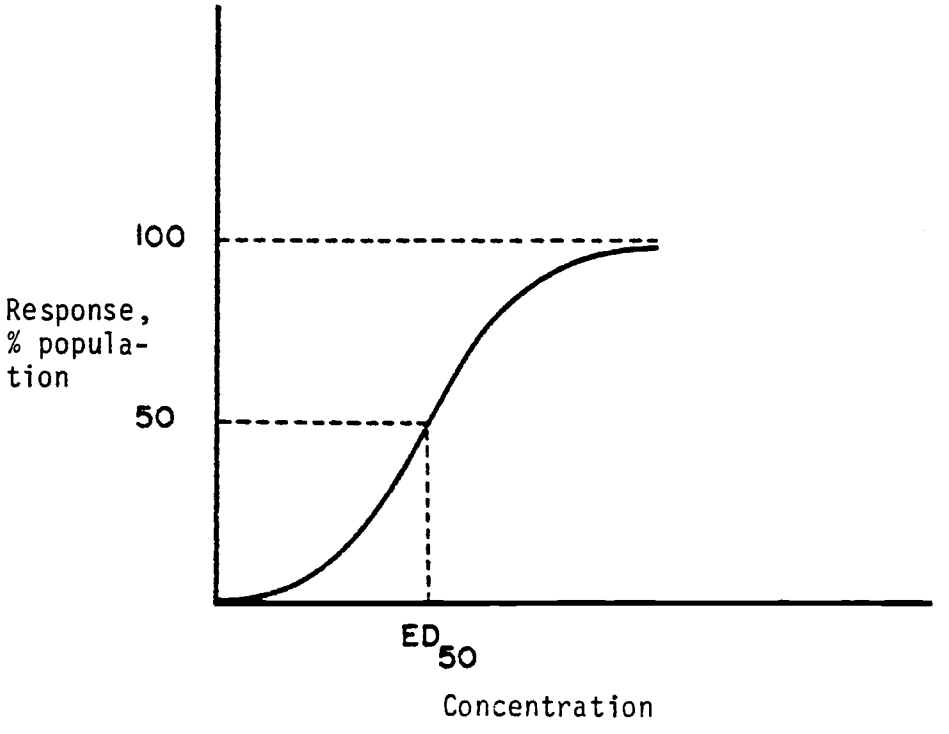

Figure 2.4. Dose-response relationship assuming normally distributed log-thresholds. ED_{50} represents the effective dose which induces cancer in 50% of the population exposed to the carcinogen.

is that it provides a reasonable fit only in the middle region of the normal curve.

For high doses, all of the models discussed, fit the experimental data equally well. For very low levels of dose, however, the use of different models could result in response levels several orders of magnitude apart.

CHAPTER III

PHARMACOKINETIC MODELS FOR CARCINOGENIC RISK ASSESSMENT

It has been well known that many chemicals that induce cancer in animal tissues are not carcinogenic themselves, but the products of their enzymatic breakdowns, i.e., their metabolites, induce the carcinogenic processes.

Some of these metabalites may interact with DNA, RNA, and proteins. It is, therefore, extremely important to study the effect of interaction of these metabolites with cells macromolecules, especially DNA, which codes for transcription of RNAs which in turn, code for translation of proteins.

The purpose of this chapter is to discuss some biological mechanisms of neoplastic formation, their implications in carcinogenic risk assessment and their inclusion in mathematical models.

3.1 DNA-Aduct Formations in Carcinogenic Processes

A cell's genetic material, the DNA, is composed of covalently and hydrogen bonded units that can react with many chemicals. Normally, those sections of DNA covalently bonded to carcinogenic metabolites would either be removed or repaired by specific enzymatic reactions known to take place frequently within cellular structures. Occasionally, these altered sections of DNA remain attached to the

DNA either due to biochemical nature of the carcinogen, or inability of cell's enzymatic mechanisms to remove or recognize the mutation.

Russel et al. (1982) concluded that the nonlinear behavior of the dose-response curve at low dose levels was a result of efficient enzymatic removal of DNA aducts (sections of DNA bonded to the carcinogen metabolite) and not a result of a decreased concentration of chemical reaching the organ.

Several investigators have shown that induced frequencies of mutations are linear functions of the DNA aducts concentration (Newbard and Brooks, 1979; Fahl and Scarpelli, 1981). Certain compounds are known to inhibit the carcinogenic process. In one experiment performed on two strains of mice, benzo[a]pyrene diol epoxide-DNA aduct formation was completely inhibited as a result of treatments by TCDD (2, 3, 7, 8-tetrachloro dibenzo-p-dioxin) (Cohen et al., 1979).

Hoel et al. (1983) suggested that it was, therefore, more meaningful to relate the incidence of tumor to the concentration of DNA aducts and not to the administered dose.

3.2 Model Derivations

Denoting the concentration of DNA aduct by d^* and the administered dose by d, following relations are assumed to exist:

$$d^* = f(d) \qquad \text{Eq. 3-1}$$

$$P_r = g(d^*) \qquad \text{Eq. 3-2}$$

where P_r denotes the incidence rate. Although g may be obtained by

various statistical models, determination of f would remain to be investigated by kinetics of the reactions leading to neoplastic induction.

In order to determine f, the mechanisms described by Figure 3.1 were used (Hoel et al., 1983). [C], in the diagram, represents the non activated chemical which would either be excreted and/or activated to form the reactive metabolite, RM. The concentration of the excreted chemical is represented by $[C_e]$. The reactive metabolite would either be detoxified by cell's enzymatic mechanism to form an inactive metabolite IM, or it could bind covalenly either to nongenetic macromolecules of the cell (CBN), or to genetic materials of the cell such as DNA (CBG). Note that all these reactions could take place simultaneously. That is, while some molecules of the reactive metabolite are being detoxified, others are involved in reactions resulting in formation of CBG or CBN.

If the metabolite-DNA aducts are formed, they may participate in DNA replication processes during cell division and result in daughter cells with genetically altered structures (RCBG). If, however, the damage is repaired by enzymatic mechanisms, no mutation results. The product of the repair reaction is symbolized by CBGR (repaired covalently bound genetic material).

In this model, (V_M^A, k_M^A), (V_M^D, k_M^D), and (V_M^R, k_M^R) represent Michaelis-Menten[1] parameters for activation, detoxification, and

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[1]For description of Michaelis-Menten kinetics see Appendix B.

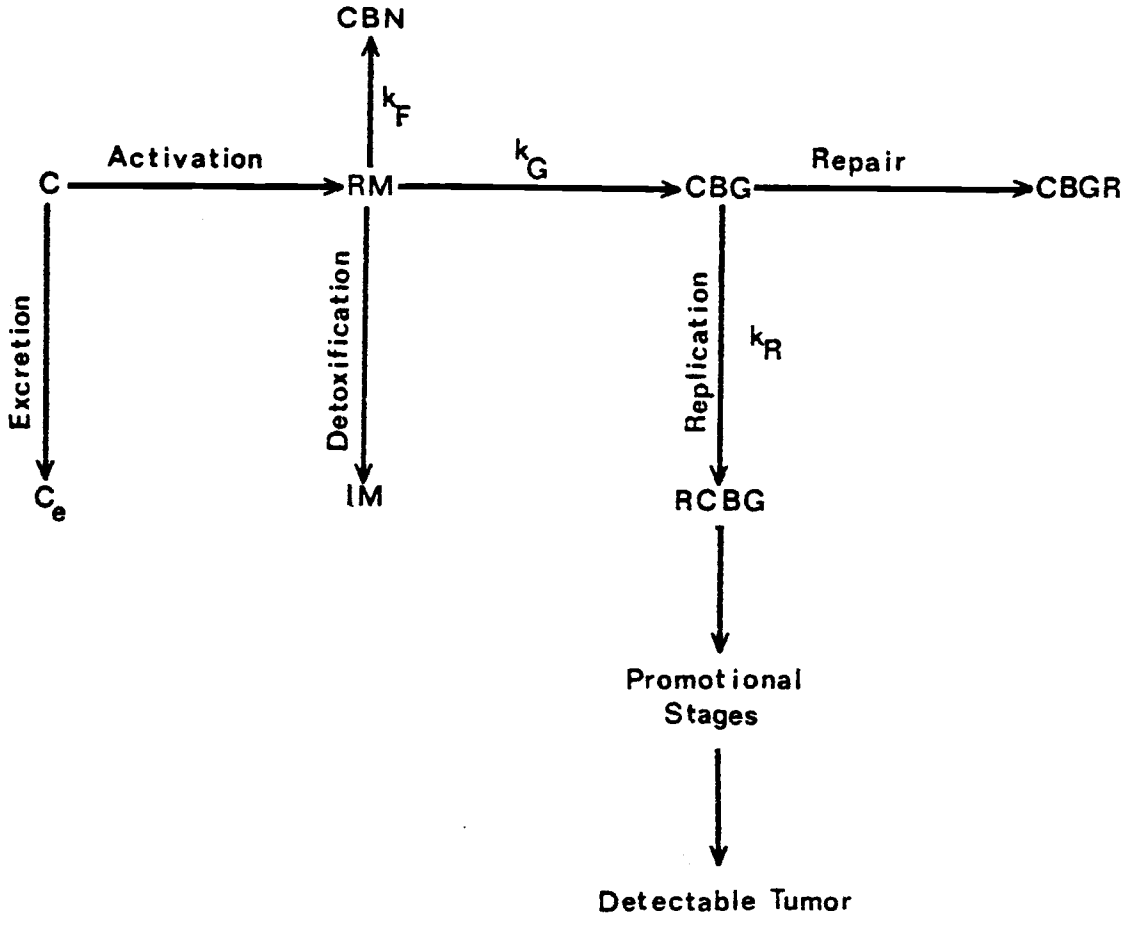

Figure 3.1. Schematic of a simple pharmacokinetic example. (from Hoel et al., 1983)

and repair mechanisms. Other reactions are assumed to follow first order kinetics with rate constants k_F, k_G, and k_R as shown on the diagram.

It is important to note that this is one of the simplest cases that can be considered for carcinogenic processes. Nevertheless, it deserves consideration since it contains a more fundamental description of neoplastic induction processes than the models previously discussed.

Kinetics of the process described by Figure 3.1 would vary for different carcinogenic metabolites. Therefore, f has to be determined for each chemical administered. This model was developed on the basis of exposure to a single chemical carcinogen. In addition, steady state conditions were assumed in the derivations of f. A special characteristic of this model is that for constant administered dose, the concentration at each stage of the diagram in Figure 3.1 may be determined by using simple mass balance equations. For example, at equilibrium conditions, assuming Michaelis-Menten kinetics for enzyme catalyzed reactions of activation, detoxification, and repair, and first order kinetics for other reaction, the following equalities hold:

Rate of formation of CBG = rate of removal of CBG

$$k_G\,[RM] = \frac{V_M^R\,[CBG]}{k_M^R + [CBG]} + k_R[CBG] \qquad \text{Eq. 3-3}$$

where the terms in brackets denote concentrations.

Also, at equilibrium

$$\frac{V_M^A [C]}{k_N^A + [C]} = k_F [RM] + k_G [RM] + \frac{V_M^D [RM]}{k_M^D + [RM]} \qquad \text{Eq. 3-4}$$

The concentration of RCBG is given by:

$$[RCBG] = k_R [CBG] \qquad \text{Eq. 3-5}$$

Since [RCBG] is proportional to [CBG], d^* is chosen to be equal to [CBG]. Special attention must be paid to the concentration of C, which may or may not be proportional to the applied dose d. [C] is thought to be proportional to d when the chemical structure of the administered agent is such that it can easily diffuse through the cell membrane. When [C] is not proportional to d, then the relationship must be obtained experimentally.

Substituting the parametric values for k's and V's in equation 3-3 and 3-4, and solving the two equations for the two unknowns [RM] and [CBG], for given values of [C], one can obtain relationships between the applied dose d and the effective dose d^*. Figure 3.2 illustrates this relationshp for vinyl chloride (Hoel et al., 1983).

Once d^* (or effective dose, [CBG]) is determined, the probability of response can be obtained from a multi-stage model of carcinogenesis. It is interesting to note that at low doses, the relationship between [C] and [CBG] is approximately linear. However, changing some of the parameters in equations 3-3 and 3-4 can have a dramatic effect on this apparent linearity. For example, decreasing

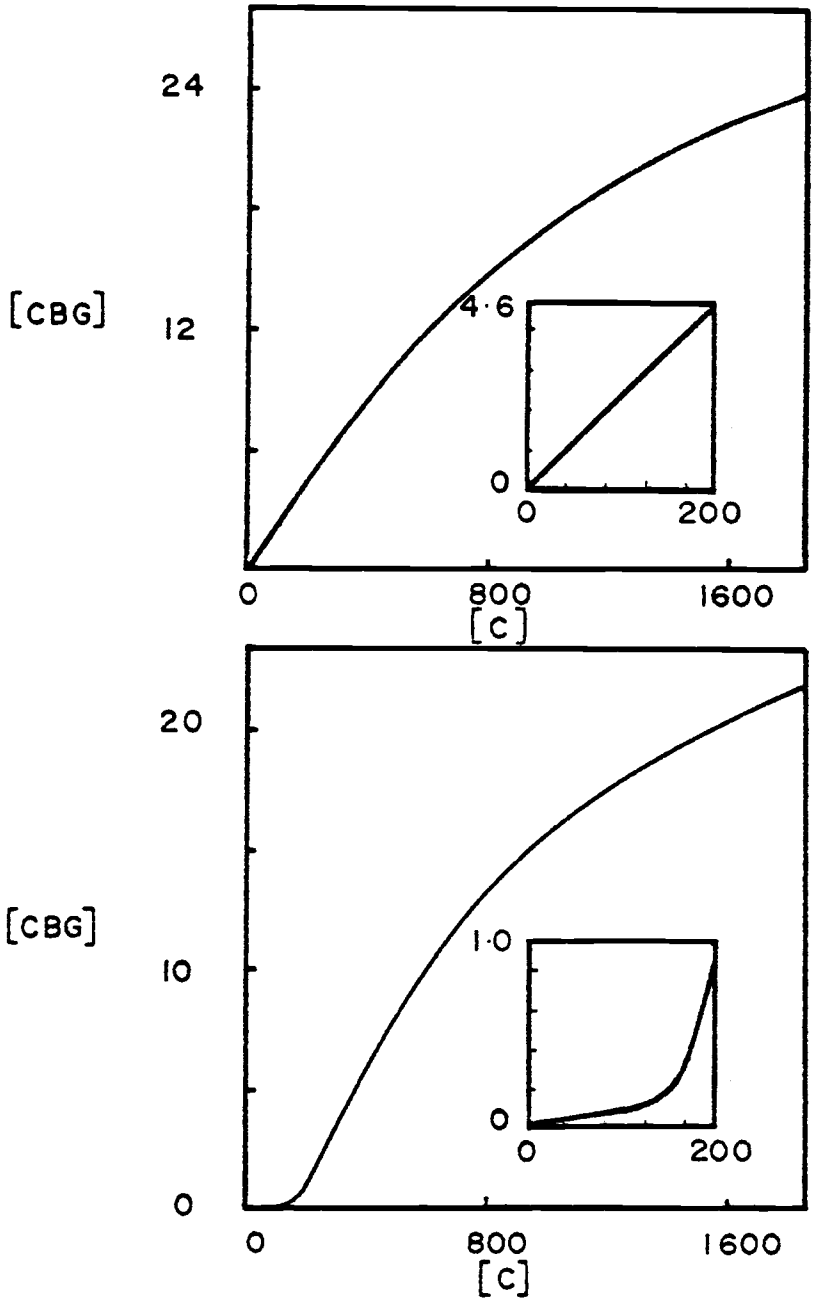

Figure 3.2. (a) Relation between concentration of carcinogen [C] and the concentration of the metabolite covalently bonded to genetic material [CBG] for vinyl chloride kinetic example (from Hoel et al., 1983). The curve is a result of equations 3.3 and 3.4 with parameters given in Table 3.1. (b) Relation between [C] and [CBG] after the detoxification system is overwhelmed by changing k_M^D from 10 to 0.1.

Table 3.1

Estimated Parameter Values for Vinyl Chloride

V_M^A (maximum velocity of activation)	5706	g/4 hours
k_M^A (Michelis-Menten constant for activation)	860	g/lit
V_M^D (maximum velocity of detoxification)	1000	g/4 hours
k_M^D (Michaelis-Menten constant for detoxification)	10	g/0.25 kg
V_M^R (maximum velocity of repair)	100	g/4 hours
k_M^R (Michaelis-Menten constant for repair)	70	g/0.25 kg
Reaction rate constants (as shown in Figure 3.1)		
k_F	50	lit/4 hours
k_G	5	lit/4 hours
k_R	100	lit/4 hours

From: Anderson et al., (1980). These are the parameters employed by Hoel et al. (1983) to produce the results in Figure 3.2.

the value of k_M^D by 100 fold and keeping the other parameters constant, would result in increased rate of detoxification at low doses. This is illustrated by curve 2 in Figure 3.3. Note how this one parameter change significantly increased the detoxification rate at low doses. With this increase of detoxification at low concentration levels, the model shifts from a near-linear relationship (Figure 3.2(a)) to a nonlinear relationship (Figure 3.2(b)). A lower detoxification rate at low doses may be expected when the enzymatic system for detoxification is overwhelmed by other carcinogen metabolites.

The present pharmacokinetic models do not include such interactions. However, this model (Hoel et al., 1983) does suggest that if the saturation of detoxification by other background carcinogen metabolites occurs, then, the relationship might shift toward a linear relationship as shown in Figure 3.2(a). That is, the rate of detoxification of the additional metabolites would be reduced due to engagement of the enzyme molecules in ongoing detoxification processes. Thereafter, the cell's ability to detoxify the additional metabolite would be decreased because of the presence of background metabolite concentrations. This would result in a more linear curve (such as one in Figure 3.2(a)) at low doses.

The pharmacokinetic model, as described by Hoel et al. (1983), failed to address the question of multiple pollutants and marginal risks. Nevertheless, the results are valuable in the sense that they indicate the absence of any "threshold" doses for the vinyl chloride

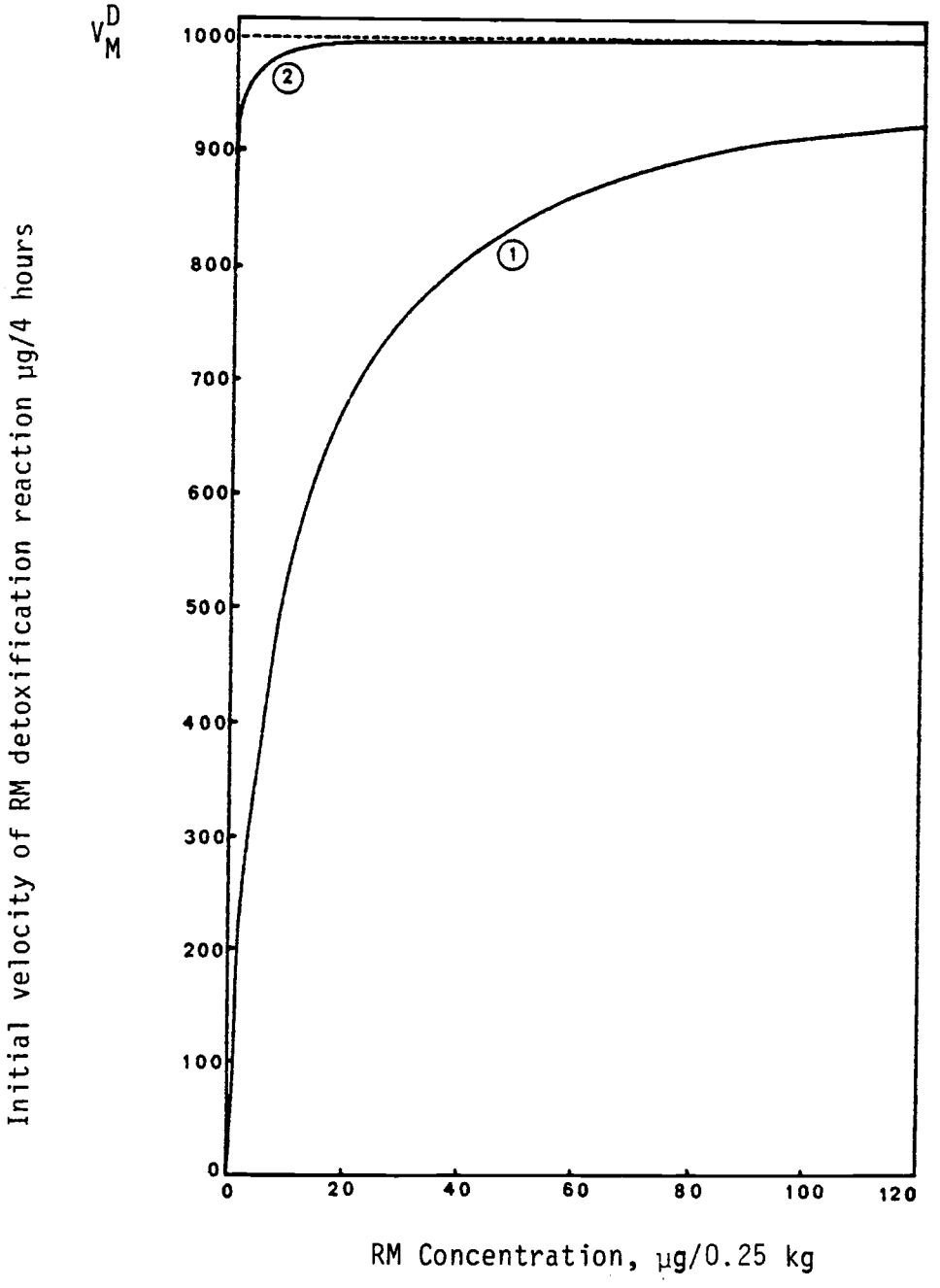

Figure 3.3. Rate of detoxification as a function of RM Concentration 1) $k_M^D = 10$; 2) $k_M^D = 0.1$.

example. In each case examined, there were no positive doses at which the probability of cancer incidence could be zero.

Cornfield (1977) developed a model for carcinogenesis resulting in a single threshold. His single exposure, steady state model required complete deactivation of carcinogen at the critical target on DNA. The assumptions were based upon the existence of at least one completely irreversible deactivation reaction. Such an assumption of irreversibility cannot be made in many biological reactions. For example, in the case of hydrocyanic acid-Thiosulfate cytochrome oxidase system, a reverse deactivation reaction exists.

Biochemical deactivation reactions take time to reach equilibrium. Under Cornfield's assumption that dose and response incidence are proportional, therefore, such thresholds cannot exist, since even one activated molecule would be sufficient to initiate the carcinogenic process. Cornfield's model would result in low dose linearity in such cases (Crump, 1978).

The assumption of a single exposure in Cornfield's model is also quite unrealistic, since exposure to environmental pollutants as well as production and degradation of deactivator, is a continuous process. If carcinogen Y is applied continuously to his model, the amount of Y-DNA complex will not be zero even under steady state conditions, and thus the argument for an absolute threshold is not supported.

CHAPTER IV

IMPLICATIONS OF EXISTING DOSE-RESPONSE MODELS ON ENVIRONMENTAL RISK ASSESSMENT FOR CARCENOGIES

The ongoing debate on dose-response relationships for carcinogenic risk assessment has given rise to questions regarding linearity of response at low doses, and marginal risk associated with introduction of a new carcinogen to the present pool of carcinogens.

None of the five models cited, addressed the subject of exposure to multiple pollutants. The dose-response relationships discussed, were developed for a single carcinogen. A more realistic model, however, would include the concept of exposure to a combination of carcinogenic agents having additive, synergistic, or antagonistic effects on one another. Humans are exposed simultaneously to more than one pollutant, some are encountered repeatedly, while the others are highly persistent and reside in biological tissues for long periods of time before they are degraded.

In this chapter, some of the important factors in quantitative carcinogenic risk assessment are discussed.

4.1. Review of the Models for Carcinogenic Risk Assessment

The concept of additivity of carcinogens is not included in any of the models discussed herein. The significance of this issue may severely undermine the foundations of the existing models' assumptions.

In general, all of the models discussed (except for the log-probit model) assume that neoplastic growth is a result of a single cell being altered at some critical target sites by one or more transitions.

The multi-hit and multistage models assume that the development period of the tumor is independent of the initial dose. This assumption may hold true in low-dose regions. However, for sufficiently large values of dose, the applicability of this assumption is highly questionable.

The multistage models of carcinogenesis such as the one developed by Armitage and Doll (1961) appear to be more realistic theoretical developments. In particular, the observation that cancer incidence rates dramatically increase with age (for human cancer), would support the general form of the age-specific incidence rate given by:

$$I(t,D) = kt^{(k-t)} \prod_{i=1}^{k} (\alpha_i + \beta_i D) \qquad \text{Eq. 4-1}$$

where

t = exposure time (equal to age for a constant dose rate)

D = dose rate

k = number of stages

The log-probit model discussed in Section 2.3 is based upon several assumptions not shared by the hit-theory models.

The log-probit model assumes the existence of a threshold for each individual in a given population. Furthermore, it assumes that the distribution of log-dose thresholds is normal. However, existence of individual thresholds is a matter that has not been supported either by the stochastic models or by recent pharmacokinetic models. The log-probit model is generally used for experimental curve fitting of biological data and only the central region of the distribution of the distribution provides a reasonable fit.

At this point in the discussion, one would realize the significance of biological mechanisms in derivations of these models. The stochastic models do not include any assumptions regarding the kinetics of mechanisms leading to genetic alterations, tumor induction, and neoplastic growth. Nevertheless, the requirement of a minimum number of "hits" on a cell's traget sites appears to be applicable to many biological situations.

The pharmacokinetic model developed by Hoel et al. (1983) incorporates some of the biochemical mechanisms that the previous models failed to address. This model may open an interdisciplinary dialogue between toxicologists, epidemeologists, mathematicians, and environmental scientists and engineers. The nature of such dialogues are essential to the success of research of this nature.

An important result obtained from the discussion of a pharmacokinetic model is the observation that increased enzymatic activity during the detoxification process (due to the presence of other carcinogenic compounds), would result in a nearly linear curve. This

curve represents the steady state concentration of the carcinogen metabolite as a function of the concentration of carcinogen. Furthermore, these relationships appear to be similar to the shape of the dose-response curves obtained by stochastic models. One, therefore, may investigate the probability of response as a function of the carcinogen metabolite-DNA concentration.

The one-hit model results in linear dose-response relationships for low doses. The multi-hit and multistage models in linear dose-response curves for k=1, but nonlinear relationships for k> 1. Unfortunately, experimental results provide no conclusive evidence for supporting any of these models in favor of the others. In addition, for very low concentrations, statistically significant results cannot be obtained from animal experiments.

The general practice regarding animal experiments is to expose fewer number of animals to higher (than environmentally occurring) concentration levels, and extrapolate the results to low concentrations using the models discussed in previous chapters.

Because these models do not support the hypothesis of "thresholds", one therefore may not assume the existence of no-effect dose levels for chemical carcinogens.

A review of 151 dose-response curves for chemical carcinigenesis in laboratory animals provided no clear indication of a threshold for any carcinogen tested (Lepkowski, 1978), with either the one-hit or the log-probit (nonlinear) models used for low-dose extrapolations.

Rall (1978) discussed the concept of "thresholds" and concluded:

> The issue is not thresholds or no thresholds; it is one of the adding a new carcinigen to a pool of present carcinogens. I would suggest, therefore, that there may well be thresholds with carcinigenic substances when given to a very clean animal in an environmentally controlled situation, that is, when there are few or no other carcinogens present; this is what the experimental oncologist tries to create in the standard laboratory animal test system - a clean animal of known and homogeneous genetic background with a well characterized diet and no known carcinigens living in sterile filtered air. The human population is different, however: the mouse doesn't smoke or breath hydrocarbons or sulfur oxides from fossil fuels, doesn't drink, doesn't take medicine, doesn't eat bacon or smoked salmon, but man does.

Jones et al. (1976) suggested the concept of "practical thresholds". He observed that the latent period for neoplastic development was dependent on initial dose for most carcinogens ranging from cyclic hydrocarbons to low level radiation. He then suggested that the following time-dose relationship existed for carcinogenic processes beyond the transformation stage:

$$t_e = t_1 \, (D_1/D_e)^{1/n} \qquad \text{Eq. 4-2}$$

where

t_e = latent period associated with D_e

t_1 = latent period associated with D_1

D_1 = initial dose

$n \simeq 3$

He then concluded that for low enough values of D_e, time-to-tumor could exceed human lifespans. In effect, "practical thresholds" would exist. Additive or synergistic interactions of a

variety of carcinogens each having its "practical threshold", were not addressed by his study. In addition, one may question the ethical justifications regarding "practical thresholds". The argument might then center over the possible transmission of altered DNA (or perhaps RNA) to future generations resulting in higher susceptibility to cancerns of various kinds.

4.2. Some Characteristics of Carginogens

The mechanisms for synergistic effects of pollutants and drugs are not fully understood (Marking, 1977). Proposed theories regarding synergistic effects include formation of carcinogenic metabolites and/or inhibition of detoxification reactions. The evidence exists that methylenedioxyphenyl compounds are metabolized by a multiple function oxidase system which also participates in oxidation of xenobiotics (Knudson, 1973).

The chemical EPN has synergistic effects on the pesticide malathion in such a way it inhibits the nonspecific enzyme carboxy esterase that detoxifies malathion (DuBois, 1978). Strong evidence exists in support of interactions between a polynuclear hydrocarbon carcinogen such as benzo [a] pyrene and croton oil. An agent in croton oil known to be phorbol myristate acetate (PMA) acts as the activator for benzo [a] pyrene resulting in increased incidence of cancer tumors (Berenblum, 1941).

Many investigators now believe in a two-stage mechanism of carcinigenesis- namely, initiation stage and promotion stage. The first

stage, requiring an initiator to alter DNA structure has been addressed in almost all of the models discussed. The mechanisms of the second stage which requires an agent - a promoter - is not fully understood, but several authors have pointed to the possibility of action during cell division (Sivak, 1972), cyclic AMP (Belman and Troll, 1973) or intracellular protease release (Trall et al., 1970). The reality of carcinogenic interactions is not well recognized in the present models.

Crump et al., (1976) showed that if carcinogenic processes induced by a new pollutant act additively with any ongoing processes, then under almost all models developed, the response would be linear for low doses. In particular, their results indicate that extra (or marginal) response due to extra dose above the background level increases quite linearlly throughout the dose range (see equation 4-11)

A second consideration regarding Crump's work is that of "spontaneous" vs. "induced" carcinogenesis. He concluded that if carcinogenic processes are considered to be initiated by mutational changes in DNA structure, then induced carcinogenesis would simply follow a similar pathway as that of spontaneous carcinogenesis.

4.3. Additive Effects of Carcinogiens

To understand additive effects, consider dose d of a particular (primary) carcinogen. Assume that the totality of background carcinogens can be divided into two groups (Crump et al., 1976). Group 1 contains all those carcinogens that induce neoplastic growth

in ways that are completely independent of the way the primary carcinogen induces cancer. Group 2 contains all those carcinogens (along with spontaneous biochemical incidents) that in some way act in conjunction with the primary carcinogen. Assume

$$I = I_1 + I_2 \qquad \text{Eq. 4-3}$$

in which I equals the total incidence of cancer, I_1 equals the incidence due to group 1 and I_2 is the incidence due to group 2. Let

$$I_M(d) = I(d) - I(o) \qquad \text{Eq. 4-4}$$

in which I_M is the marginal incidence of dose d (the primary carcinogen), I(d) is the total incidence with dose d and I(o) is the total incidence without dose d. Let

$$I_2 = H(D) \qquad \text{Eq. 4-5}$$

and

$$D_o = \sum_{j=1}^{M} \rho_j \, d_j \qquad \text{Eq. 4-6}$$

in which, D_o is the total effective dose in group 2 in the absence of the primary carcinogen, D is the total effective dose in group 2 when the primary carcinogen is present, dj is the dose level of carcinogen j in group 2, M is the total number of carcinogens in group 2, and ρ_j is an unknown constant. Note that if synergistic or antagonistic reactions occur, a more complex relationship would apply.

Assuming an additive effect for the primary carcinogen in group 2, one obtains the following:

$$D = D_0 + \rho d \qquad \text{Eq. 4-7}$$

where ρ is a constant of proportionality.

Combining equations 4-3, 4-4, 4-5, and 4-7 results in the following expression:

$$\begin{aligned} I_M(d) &= [I_1 + H(D_0 + \rho d)] - [I_1 + H(D_0)] \\ &= [H(D_0 + \rho d)] - H(D_0) \end{aligned} \qquad \text{Eq. 4-8}$$

Using a Taylor series expansion of 4-6 one obtains:

$$I_M(d) = [H(D_0) + H'(D_0)\rho d + H''(D_0)\frac{(\rho d)^2}{2!} + ...] - H(D_0) \qquad \text{Eq. 4-9}$$

or

$$I_M(d) = H'(d_0)\rho d + \frac{H''(D_0)(\rho d)^2}{2} + ... \qquad \text{Eq. 4-10}$$

If $I_L < H(D_0) < I_U$, where I_L and I_U are incidence quantities shown on Figure 4.1, then $H(D_0)$ falls within the near linear portion of the Incidence-dose curve and the higher order terms can be neglected.

Equation 4-8 reduces to

$$I_M(d) = \rho H'(D_0)d \qquad \text{Eq. 4-11}$$

Thus, the marginal (extra) incidence is a linear function of dose d.

We can say that d has a response (incidence) additive effect with group 1 carcinogens and a dose (concentration) additive effect with group 2 carcinogens. If dose d has other than an additive effect within group 2, equation 4-5 should be modified to

$$D = D_0 + \rho d + h(d_1, d_2, ... d_M, d) \qquad \text{Eq. 4-12}$$

in which h is the non-additive increase in the effective dose, D.

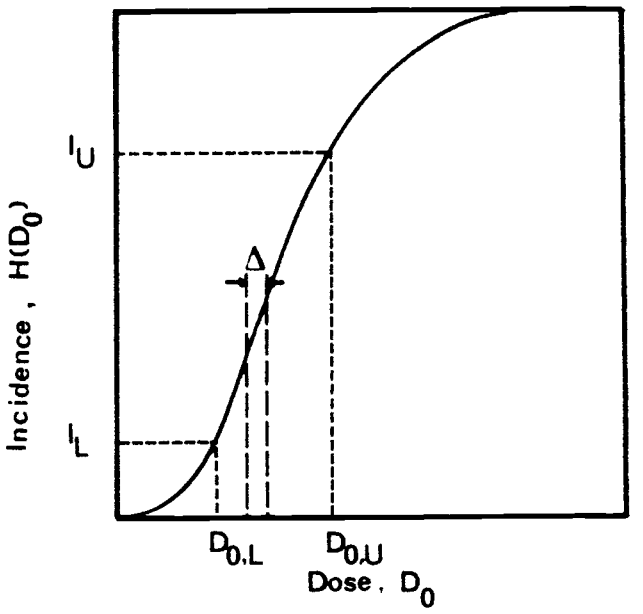

Figure 4.1. Diagram of a Nonlinear Dose-Response Relationship. An incremental increase of dose, Δ, in excess of background results in a linear increase in response.

If h has a positive value, then d is said to have a synergistic effect. If he has a negative value, d is said to have an antagonistic effect.

Humans show a significant level of spontaneous response (approximately 20% death rate) due to already existing carcinogenic agents. The total incidence rate including undetected cancers is higher than the death rate due to cancer. The incidence rate of any mpechanistically related group of cancers is, obviously, less than the total cancer incidence rate. Thus, the assumption of a background incidence rate and the presence of congruent carcinogens must be considered. As previously discussed, such considerations may support a linear dose-response relationship for the addition of a carcinogen even when nonlinear models are employed (see Figure 4-1.)

4.4. Relationships Between Dispersal and Total Population Incidence

Most of the debate over the linearity on nonlinearity of the dose-response relationship is concerned with the extrapolation of risk estimation to low doses. The nonlinear models generally yield substantially lower risks at a low concentration than does the linear model. Similarly, nonlinear models allow for higher doses (concentrations) for a given level of risk than do linear models. There is, however, an equally important issue, hereafter called the dispersal issue, that the literature has not addressed.

For the purpose of this discussion, dispersal will refer to the degree to which a given mass of a carcinogen is distributed through-

out a population. A high dispersal means that the mass is evenly distributed and each person receives a relatively equal dose. A low dispersal means that the mass is concentrated within the population and thus a few individuals receive a high dose while the majority receive a much lower dose. The dispersal issue concerns the total response of the entire population as a function of dispersal.

If one assumes a nonlinear dose-response relationship for a carcinogen, the total response in the entire population can be reduced, by maximizing dispersal(2) (See Appendix C). However, given the linear assumption, the total risk is equal if a large population receives a low dose or if a small population receives a high dose (all else being equal).

If the effects of synergistic interactions are greater than antagonistic interactions, higher levels of dispersal may lead to higher levels of total response. High levels of dispersal may then be undesirable. Note that dispersal must not be confused with transport. If pollutants are transported to areas where population density is very low, then the risk of cancer would be decreased by less exposure (or contact).

The effect of dispersal upon the total incidence of cancer in a population thus depends upon the linearity or nonlinearity of the marginal dose-response relationships and the relative degree of synergism and antagonism. Higher levels of dispersal may be either

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(2) This statement assumes no synergistic or antagonistic interactions. Such interactions are included later in the Discussion.

beneficial or harmful. Thus, debates over dose-response relationships and background interactions should involve more than the issue of low dose extrapolation. An additional issue is the harm or benefit associated with dispersal.

The paradigms of environmental science, engineering and management are in general based upon the implicit assumption that dispersal is desirable. This assumption is most obvious in the technological efforts to increase dispersal (i.e. ocean outfalls, difusers, smoke stacks, etc.) Less obvious, but probably more significant, is the practice of relating environmental risk to the concentration of pollutants (usually mass per unit volume). It is commonly assumed that acceptable risk may be attained by keeping the concentrations of pollutants below some stated level. Under this assumption, a problem is identified when a concentration exceeds some prescribed level. Risks are managed by defining standards for maximum permissible concentrations. All of these notions implicitly assume that high dispersal is good because it leads to a reduction of maximum concentrations. Debate then centers over the appropriate levels of maximum permissible concentrations. The desirability of dispersal itself is seldom addressed. Dispersal is presumed to be desirable.

In effect the presumed desirability of dispersal assumes a nonlinear dose-response relationship. The notion of a threshold concentration (a special case of nonlinearity) below which risk is essentially zero is often presumed. Under such an assumption, dispersal is desirable, particularly if maximum concentrations are

reduced to below threshold levels. For example, if concentration is normally distributed over a population of N_o. Individuals with individual i receiving the maximum concentration. Then assuming the existence of a threshold for carcinogenic risk evaluations, one would want to decrease C_{max} to a prescribed level, C_T (see Figure 4.3). The implication of the linear carcinogenic model, however, would be to minimize the average concentration that a person encounters over time, that is, to minimize

$$C_{avg} = \frac{C_1 + C_2 + ... + C_{N_o}}{N_o} \qquad \text{Eq. 4-11}$$

where C_1, C_2,..., C_{N_o} are concentrations encountered by individuals 1, 2, ..., N_o, per unit time. In the case of carcinogens, however, the scientific community has not accepted the nonlinear marginal dose-response relationship as the universal norm. A linear relationship appears to be accepted by many toxicologists, particularly where a background level of cancer is found and multiple carcinogens are likely to be present. The literature does not support a universal assumption of nonlinear dose-responses or thresholds under such conditions. Thus, by presuming dispersal to be desirable, broad areas of environmental science, engineering, and management are implicitly assuming a relationship (nonlinear dose-response) that has not been accepted within the disciplines of science that deal with this relationship. The debates over dose-response relationships appear to involve more than the toxicological literature indicates. There

appears to be a serious interdisciplinary dysfunction and thus there is a grave need for a higher level of interdisciplinary dialogue.

4.5 A Proposed Experiment for Multiple Pollutant Risk Assessment

Dispersing pollutants would result in more carcinogens being in contact with each other within any given environment, whereas low dispersal (or concentrating a pollutant in a particular area) would prevent many synergistic or additive reactions (which are due to presence of other pollutants) to take place.

A conceptual experimental design is presented which might test for a general relationship between dispersal and total population incidence. Given

1. An equitoxic[3] or equicarcinogenic dose, D_0 for each of N_0 different chemicals (carcinogens or suspected carcinogens).
2. A N_0 different environments (containers)containing equal numbers of test organisms
3. N_0 dose which induces cancer in the same percentage of all laboratory animals exposed for different pollutants.

Two test runs would be compared.

Test run 1: Each environment would receive a dose D_0 of one and only one chemical.

Test run 2: Each environment would receive a dose D_0/N_0 of each and every chemical

The total incidence of tumor would be measured for each test run. If the total incidence of test run 1 was greater than test run

[3]A dose which indures cancer in the same percentage of all laboratory animals exposed for different pollutants.

2, an inverse relationship between total incidence and dispersal would be implied. Contrary results would imply a direct relationship between total incidence and dispersal. As N_0 is increased, the total number of organisms would be kept constant (the number per separate environment would decrease). At higher levels of N_0 some tendencies might emerge that would indicate a general relationship between total incidence and dispersal. Dispersal would be given by N_0-1 (the number of equal volumes over which each chemical is dispersed). Experiments of this nature were not cited in literature reviewed for this study, and results, if exist, are not included in discussions.

CHAPTER V

CONCLUSION

Several dichotomous response models for carcinogenesis were reviewed and their implications for low dose estimations were examined. Such models are mathematical translations of fundamental concepts held (explicitly or implicitly) by those employing them. This study sought to examine these fundamental concepts with regard to problems faced by environmental engineers, scientists, and regulatory agencies. The following observations were made

1. None of the models reviewed, displayed an absolute threshold (no response level), to a given population, however, all but the one hit model could potentially display a nonlinear (concave up) dose-response relationship at low dose regions. This nonlinearity may lead to extremely low responses at low dose levels, therefore providing an "effective threshold". Arguments for existence of thresholds appear to be most applicable to natural carcinogens for which detoxification and repair mechanisms have likely evolved. For synthetic carcinogens, arguments for existence of no-effect dose levels appear to be less compelling.

2. The issue of chronic exposures to multiple pollutants was typically excluded from formal developments of the models and discussions of additive effects were often vague. Synergistic and antagonistic effects were even less frequently discussed.

3. When a background of congruent (similarly acting) carcinogens is present, a "concentration additive" effect may occur with the addition of a new carcinogen. Then, the marginal response for the new carcinogen may approach a linear relationship with dose (or concentration) even when nonlinear models are employed.

4. The debate over the linearity or nonlinearity of dichtomous response relationships has been primarily concerned with extrapolation of response or incidence data to low dose values. A separate concern identified in this study, treats the effects of pollutant dispersal upon total incidence of cancer within a population. It was indicated that if a linear dose-response relationship occurs (at the margin) for a given group of carcinogens, then, dispersal of pollutants from this group for the purpose of decreasing maximum concentrations, is not likely to have beneficial effects on the overall population. Dispersal, however, is presumed to be an effective means of environmental quality control. This presumption may not be justified for an important class of carcinogens.

5. The dose-response models reviewed herein are not likely to clarify the effect of dispersal upon the total incidence of cancer within a population. Without revision of their basic assumptions, they are not capable of treating the net effect of dispersal of multiple carcinogens. Furthermore, the standard animal carcinogenicity experiments (which reflect similar assumptions) are not likely to resolve the "linearity" vs. "nonlinearity" (threshold) debates, particularly in the presence of a background of multiple, low level

carcinogens. As a result, these standard tests are not likely to resolve the controversies concerning the estimation of risk to a total population given multiple synthetic carcinogens.

6. An outline of an alternative experimental approach was presented from which the effect of dispersal upon the total tumor incidence rate of a population exposed to multiple carcinogens might be examined.

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APPENDICES

APPENDIX A

GAMMA PROBABILITY LAW

Consider a Poisson process beginning at dose zero. Let D_k be the dose corresponding to the kth event or "hit", where $k \geq 1$. If D is any fixed positive number such that $D_k > D$, one may state that there exists a random variable X, such that $D_k \geq D$ is equivalent to the event $X \leq k-1$, and X is the number of hits occurring in the dose interval (0,D). The above statement is justified since the dose at which the kth hit occurs can exceed D only if there are no more than k-1 events in the interval (0,D). X is the Poisson variable with parameter $\Gamma = \lambda D$. One may write the following expressions

$$P(D_k > D) = P(X \leq k-1)$$

$$= \sum_{i=0}^{k-1} \frac{(\lambda D)^i}{i!} e^{-\lambda D} \qquad \text{Eq. A-1}$$

The distribution function for the dose of the kth hit, D_k is

$$F_D(D) = P(D_k \leq D) = 1 - P(D_k > D)$$

$$= \sum_{i=0}^{k-1} \frac{(\lambda D)^i}{i!} e^{-\lambda D} \qquad \text{Eq. A-2}$$

D_k is the Erlang random variable with parameters k and λ. Rearranging Eq. A-2 and differentiating, one will obtain the following expression:

$$\frac{d}{dD}\left[F_{D_k}(D) - 1\right] = \frac{d}{dD}\left[-\sum_{i=0}^{k-1} \frac{(\lambda D)^i}{i!} e^{-\lambda D}\right]$$

$$= \frac{d}{dD}\left[-e^{-\lambda D} - De^{-\lambda D} - \frac{(\lambda D)^2}{2!} e^{-\lambda D} - \ldots - \frac{(\lambda D)^{k-1}}{(k-1)!} e^{-\lambda D}\right]$$

$$= \lambda e^{-\lambda D} - \lambda e^{-\lambda D} + \lambda^2 De^{-\lambda D} - \lambda^2 De^{-\lambda D} + \frac{\lambda^3 D^2}{2!} e^{-\lambda D} - \ldots - \frac{\lambda^{k-1} D^{k-2}}{(k-2)!} e^{-\lambda D} + \frac{\lambda^k D^{k-1}}{(k-1)!} e^{-\lambda D}$$

$$= \lambda \frac{k D^{k-1}}{(k-1)!} e^{-\lambda D} \qquad \text{Eq. A-3}$$

Defining the gamma function

$$\Gamma(n) = \int_0^\infty U^{n-1} e^{-U} dU \qquad \text{Eq. A-4}$$

and considering that

$$\Gamma(n) = (n-1)! \quad \text{for } n = \text{integer},$$

one may write the following expression by a change of variable $U = \lambda x$, $dU = \lambda dx$.

$$\Gamma(n) = \int_0^\infty \lambda^n x^{n-1} e^{-\lambda x} dx$$

So that

$$1 = \int_0^\infty \frac{\lambda^n x^{n-1}}{\Gamma(n)} e^{-\lambda x} dx$$

Therefore

$$f_X(x) = \frac{\lambda^n x^{n-1}}{\Gamma(n)} e^{-\lambda x} \qquad \text{Eq. A-5}$$

is a density function and defines the gamma probability law with parameters n and λ. Note that for n=k, a positive integer, this is equivalent to the Erlang density function.

APPENDIX B

MICHAELIS-MENTEN KINETICS

Suppose that the enzyme E and the substrate S react in the following manner to yield the product P:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} P \ldots . + E \qquad \text{Eq. B-1}$$

where ES is the enzyme-substrate complex. This complex must form for the reaction to take place. The k's in Eq. B-1 represent the reaction rates. One can then state that the rate of overall reaction must be related to the concentration of ES, because for the reaction to occur, the complex ES must be formed. One, therefore, may wish to determine the concentration of ES. At steady state:

Rate of formation of ES = Rate of removal of ES

Assuming that the reaction in Eq. B-1 is an elementary reaction,

$$k_1 [E] [S] + k_4 [E] [P] = k_2 [ES] + k_3 [ES] \qquad \text{Eq. B-2}$$

Dividing by E and rearranging the results, the following is obtained:

$$\frac{[E]}{[ES]} = \frac{(k_2 + k_3)}{k_1[S] + k_4[P]} \qquad \text{Eq. B-3}$$

Assuming that $k_4 [P] \ll k_1 [S]$ and defining the total amount of enzyme as $E_t = E + ES$, the following expression can be derived,

$$ES = \frac{[E]_t\,[S]}{(k_2 + k_3)/k_1 + [S]} \qquad \text{Eq. B-4}$$

If it is assumed that the rate of reaction, V, is proportional to [ES], then the maximum rate of reaction, V_{max} will occur when all of the enzyme is present as $[ES] = [E]_t$. Setting $K_m = (k_2 + k_3)/k_1$, one can obtain the following:

$$V = \frac{V_{max}\,[S]}{K_m + [S]} \qquad \text{Eq. B-5}$$

Plotting V versus S using the above equation, one can obtain a rectangular hyperbola such as the graph in Figure B.1. K_m is called the Michaelis-Menten parameter and Eq. B-5 is the Michaelis-Menten equation.

Note that the graph in Figure B.1 is plotted for the sucrose hydrolysis reaction:

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

(See Snoeynik and Jenkins, 1976).

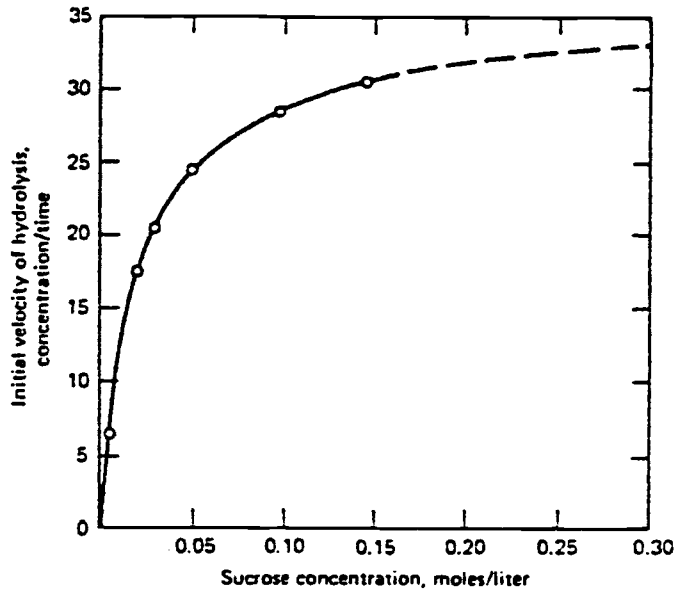

Figure B-1. Rate of Sucrose Hydrolysis by Yeast Saccharase as a Function of Substrate Concentration.

(from Snoeyink and Jenkins, 1980).

APPENDIX C

AN ILLUSTRATIVE EXAMPLE OF DISPERSAL EFFECT

To illustrate the effect of pure dispersion with different dose-response relationships a simple example is provided in Table C.1 and Figure C.1 (Bella, 1979). In this simple example two dilution conditions are examined: 1) a low dilution condition in which 3 mg/lit of a pollutant are located in one unit volume and 2) a higher dispersion condition in which 1 mg/lit of a pollutant is located in 3 unit volumes. Both conditions have the same total mass of the pollutant. The second condition has a relative dispersal of 3 with respect to the first condition. A relative indicator of the total environmental incidence, ER, is provided in column 6 of Table C1. This simple example illustrates that dispersion decreases ER with a highly non-linear relationship. Dispersion has no influence upon ER for the linear relationship.

TABLE C.1 ILLUSTRATIVE EXAMPLE OF DISPERSAL EFFECT

Figure	Relative Dispersal	Dose/ unit Area	Density of Incidence	Area of Pollutant Field	Total (a) Incidence (ER)
C.1(a)	low	4	3.5	1	3.5
C.1(a)	high	1	0.1	4	0.4
C.2(b)	low	4	5.0	1	5.0
C.2(b)	high	1	1.25	4	5.0

(a) Total Incidence = (Incidence Density) x (Area of Pollution Field)

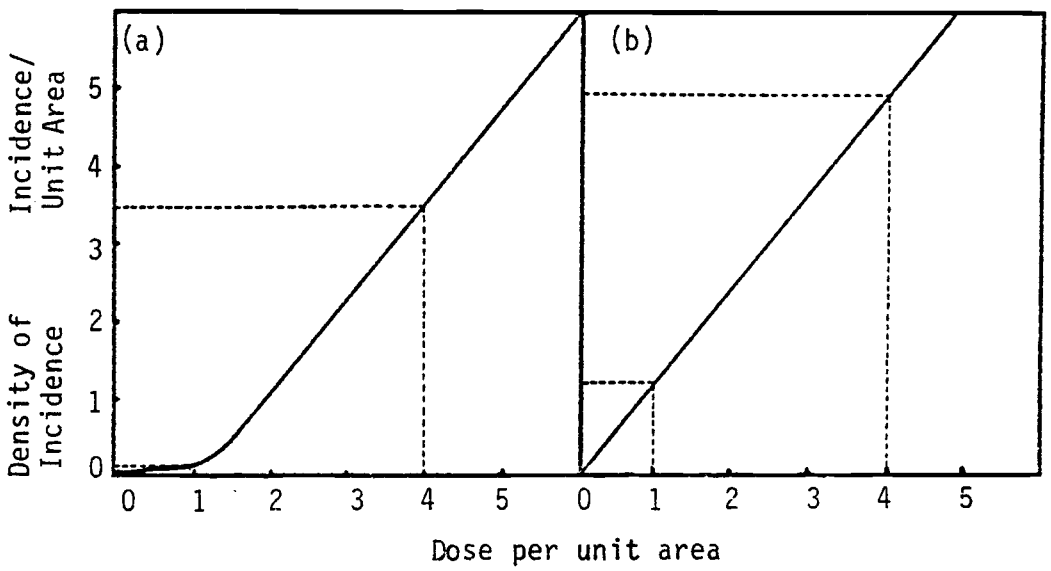

Figure C.1. Illustration of dispersal effect for two different concentrations - incidence density relationships. (See Table C.1 for explanation).