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MODELS AND METHODS IN THE RISK ASSESSMENT OF CHEMICAL
CARCINOGENS

by

DUNCAN JAMES MURDOCH, B.Math, M.Sc.

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy
Department of Mathematics and Statistics

Carleton University

Ottawa, Ontario

March 28, 1988

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ABSTRACT

Linear compartmental models are commonly used in the description of the transport and transformation of chemical carcinogens in vivo following external exposure or dosing. The general solutions under constant and variable dosing are presented. A simple formula relating the area under the concentration-time curve to the area under the dose-time curve is derived.

One of the problems in statistical analysis of these models has been the lack of a single parametrization which allows for resonance (multiple eigenvalues) and oscillation (complex eigenvalues) and at the same time provides a numerically and statistically well-behaved transformation from parameters to predictions. By pairing eigenvalues and exploiting relations between ordinary and hyperbolic trigonometric functions such a parametrization is developed. Compared to the standard sum of exponentials parametrization, it is shown to reduce the parameter effects curvature in near-resonant situations, and to smoothly extend it to allow oscillatory behaviour.

The Michaelis-Menten equation allows extension of linear kinetic models to the case of saturable transformations. Algorithms for the determination of steady-state solutions in complex networks are presented. A linear approximation to saturable kinetics is shown to hold at low doses.

Once the delivery of a carcinogenic dose to susceptible tissues is well described, it is necessary to estimate the resultant risks by a dose-response model. Sufficient conditions are developed so that a population consisting of a mixture of individuals or subpopulations with low-dose linear dose-response curves will be low-dose linear with low-dose slope being the average from the mixture. It is also shown that a mixture of various low-dose shapes will have a low-dose shape of order at least as low (i.e. increase at least as fast) as any part of the mixture.

Actual human exposures to carcinogens vary over time, in contrast with typical laboratory experiments which strive to hold the dose rate constant. A simple representation of the cumulative hazard function under time-dependent dosing patterns is derived and applied to the Armitage-Doll multistage and the Moolgavkar-Venzon-Knudson two-stage birth-death-mutation models of carcinogenesis.

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LIST OF SYMBOLS

The following symbols are used in the text with consistent meanings. Other symbols vary in meaning according to the context.

n	The number of compartments in a system.
x	The $n \times 1$ state vector of a compartmental system, representing either concentrations or amounts of a substance in each compartment.
$\dot{x}$	The $n \times 1$ vector of derivatives of x with respect to time.
x_i	Component i of the vector x . In general, subscripts are used to indicate vector or matrix components.
v_{ij}	The velocity of flow from compartment i to j .
k_{ij}	The linear coefficient of the flow from compartment i to j .
a_{ij}	The saturability coefficient of the flow from compartment i to j .
k_{ijm}	The linear coefficient of the flow on path m from compartment i to j .
a_{ijm}	The saturability coefficient of the flow on path m from compartment i to j .
R	The $n \times n$ transfer matrix for a compartmental system. Element R_{ji} describes the flow from compartment j to i .
$d(t)$	The $n \times 1$ vector of external doses input to each compartment.
d	The dosing function $d(t)$ in the case where it is independent of time.
$\text{Exp}(A)$	The matrix exponential function.
$\text{diag}(\dots)$	A square matrix constructed with the arguments of diag as submatrices on the diagonal.
m_i	The order of the i^{th} eigenvalue of R .

λ_i The i^{th} distinct eigenvalue of R , except in Chapter 6, where λ_1 represents the transition rate between stages in the cancer models.

C The complex numbers.

J The Jordan normal form of R .

J_i The i^{th} diagonal submatrix of J .

ρ_i The eigenvalue corresponding to J_i .

i The imaginary $\sqrt{-1}$.

Chapter 1.

INTRODUCTION

1. Aim of Thesis

Several problems concerning mathematical models and statistical methods involved in the risk assessment of chemical carcinogens at low doses will be considered in this thesis. Mathematical rigour will be maintained throughout, and practical application of the results will always be attempted.

2. Outline of Thesis

This thesis consists of 7 chapters. This first introductory chapter presents the aim of the thesis, this outline, and a review of the existing literature on the topics considered in the succeeding chapters.

Chapters 2, 3, and 4 are concerned with compartmental pharmacokinetic models. Pharmacokinetic models in general deal with the movement and transformation of drugs and other chemicals within living animals or humans. Compartmental pharmacokinetic models are those which model the flows and

changes as movements among a group of discrete compartments. Compartments are assumed to be internally homogeneous, so that the complete distribution of the chemical at a given point in time can be summarized by the amounts or concentrations of the chemical in each of the compartments. For example, a substance which undergoes no metabolic changes in the body could be modelled with separate compartments for each physical tissue in the body to which it is transported. Often many different tissues in the body act in the same way, so that they can be grouped into a single compartment to simplify the model. If the substance exists in two forms in the body with different physical or chemical properties, it may be better to include separate compartments representing the same physical space but the two different forms of the chemical.

Chapter 2 discusses linear compartmental systems. The general linear compartmental system obeys the system of linear ordinary differential equations given by

$$\dot{x} = R x + d, \quad (1.2.1)$$

where x is the state vector, representing concentrations or amounts of chemicals in each compartment, R is a transition matrix, and d is a dosing or input vector. This model in various forms is in very common use in pharmacokinetic modelling.

The general solution to equation (1.2.1) is expressed in Chapter 2 in terms of the initial values $x(0)$, the dosing function, and two matrices determined by R - a generalized inverse and a matrix $R^\perp$ chosen so that $R+R^\perp$ is invertible. The chapter first presents the solution in the special case of a homogeneous system, then generalizes this to the case of constant input and finally develops the general solution. Although the general solution is well-known, a non-standard development is presented here both to introduce the concepts and the notation and to facilitate the development of the formula for the area under the curve (AUC) in the last section.

The AUC for a particular compartment is defined as the integral of the concentration in that compartment over time. Simple models of toxicity relate the level of toxicity to the AUC. Based on the general solution, the last section of Chapter 2 develops a formula for the AUC under an arbitrary dosing scenario. When dosing is always received via a single route with level varying over time, it is shown that the AUC is simply a multiple of the area under the dose versus time curve. This is particularly important in risk assessment, as it allows complicated dosing scenarios to be treated very simply.

The general solution of the linear system contains terms consisting in part of exponentials of multiples of the eigenvalues λ_i of the transfer matrix R . In general R may have multiple eigenvalues or complex eigenvalues, corresponding to resonance and (possibly damped) oscillatory behaviour. Historically it has been assumed that all eigenvalues are real and distinct, in which case solutions to the model can be parametrized simply as a linear combination of exponential terms $e^{\lambda_i t}$. It is shown in Chapter 3 that solutions with multiple eigenvalues lie on the boundary of this solution space, and separate it from solutions with complex eigenvalues. A re-parametrization in which the cases of real eigenvalues (single or double) and complex eigenvalues (single pairs only) lie in the interior of the parameter space is introduced. Advantages and disadvantages of this parametrization are explored. In it solution vectors vary as continuous functions of the parameters. It is better conditioned as the boundaries of the older parametrization are approached, and has smaller parameter effects curvature in the same area. It is also nearly complete, in the sense that all possible solutions of the general system are either interior to it or are on its boundary. Finally, it retains the partial linearity property of the sum of exponentials form.

Chapter 4 considers non-linear compartmental models based on the Michaelis-Menten function. This simple function describes rates of flow through saturable paths, by increasing linearly at low concentrations yet remaining bounded in magnitude. Systems containing saturable kinetics present many practical difficulties to mathematical modellers, mainly because no general solution comparable to that for linear systems exists. A simple algorithm for computing the steady-state solution in systems containing no loops is presented first in Chapter 4. For systems with few saturable steps, analytic results are possible. For more general systems, an iterative solution based on successive approximations to linear systems is also given. This is shown to converge provided the transfer matrix is invertible.

The Michaelis-Menten function has been invoked in the risk assessment literature (Cornfield, 1977) as a justification for threshold or low-dose sublinear dose-response models. A formal proof is given in Chapter 4 that the concentrations occurring in all compartments in a Michaelis-Menten system will be linear functions of the applied dose at low levels.

Chapters 5 and 6 are concerned with some binary dose-response models used in modelling carcinogenesis. These models attempt to predict the probability of cancer developing given the dose of a carcinogenic substance. With environmental carcinogens the doses and the probabilities are generally very small. The central issue considered here is the behaviour of the models at low doses.

Many traditional models of toxicity, such as the probit model, predict a very slow increase in risk at low doses. Models which incorporate linear increases may thus predict much higher risks at low doses than low-dose sublinear models, and special interest has been given to the question of low-dose linearity. Chapter 5 presents two results concerning mixed populations. The first one shows that a mixture of low-dose linear dose-response curves remains low-dose linear with slope being the expected slope from the mixture, provided the expectation exists and the increase in dose-response at higher doses is not too large. The second result is that a mixture of dose-response curves with different orders (shapes) at the origin has order less than or equal to the essential infimum of the orders in the mixture. An example illustrating both propositions is given.

A second major issue in dose-response modelling is the effect of dosing that varies over time. In many cases exposure to carcinogens is in the form of infrequent large exposures. For example, methylene chloride, a constituent of some forms of paint remover, has been shown to be a carcinogen in laboratory animals. Most people who use it non-professionally do so only for small projects, but they may be exposed to large concentrations because of their close proximity to the work. Chapter 6 presents a simple method of comparing risks from variable exposure to those from constant exposure, and applies it in detail to two common biologically-based models of carcinogenesis. In the case of the Armitage-Doll multistage model, risks can be higher if doses occur at particularly vulnerable times, but the degree of increase is limited by the number of stages in the model. In most practical cases this will be fairly small. On the other hand, with the two-stage birth-death-mutation model of Moolgavkar, Venzon and Knudson, the degree of increase depends on the rate of replication of intermediate cells in the carcinogenic process, and may have no upper limit.

3. Review of the Literature

Chapter 2 presents a development of the well-known solution to the linear compartmental system. With the exception of the last section which derives the formula for the area under the curve, the results may all be found in Brauer et al (1970) and Jacquez (1985).

In the past decade, most research into linear compartmental models has appeared in Mathematical Biosciences, and the more recent papers below all appeared in that journal. Much current research in this area has concentrated on extending the results of Hearon (1963), who showed several bounds on the achievable eigenvalues in compartmental systems. Walter (1984) derived upper and lower limits on the sum of squared deviation of the eigenvalues of compartmental systems based on their graph-theoretic structure. Walter (1985) determined conditions under which the eigenvalues can have imaginary parts.

Another area of research has been the problem of structural identifiability of the models, i.e. determining the structure based on observation of the system. Delforge (1977) showed that this problem is inherently as difficult as the problem of determining the number of solutions to a

general nonlinear equation, and remains unsolved. Eisenfeld (1979) presented several methods based on concepts from control theory for determining the identifiability of particular systems. Fagarasan and DiStefano (1986) discuss the problem that only a lower bound on the number of compartments in the underlying system can be determined by counting the eigenvalues that are observable.

Examples of the calculation of the area under the curve for particular models are common (e.g. Gibaldi and Perrier, 1975, page 36), but expressions of the general form of it as given in section 2.5 do not seem to have appeared previously.

The problem of statistical estimation of the parameters of a linear compartmental system which may include eigenvalues with imaginary components seems to have been ignored. Among the standard texts, Jacquez (1985) and Gibaldi and Perrier (1975) do not mention imaginary components, and Godfrey (1983) does not consider their statistical estimation. Wang (1977) considered the problem of multiple eigenvalues and developed a graphical technique for parameter estimation there.

The original development of the Michaelis-Menten model

took place over 70 years ago (Michaelis and Menten, 1913). Since then the essential intractability of the differential equations underlying it has retarded progress in its development. Recently Kostova (1985a,b) has described the asymptotic behaviour of solutions to Michaelis-Menten systems. Results anticipating those in Chapter 4 appeared in Krewski et al (1984), Krewski et al (1986), and Murdoch et al (1987).

While mixture models have a long history, consideration of their low-dose behaviour is a fairly recent phenomenon. Brown (1976) and Hoel (1985) each present examples of mixtures in which the population dose-response curve has a different shape than that of any of the constituent individuals. Krewski et al (1986) gives slightly weaker versions of the results in Chapter 5.

Armitage and Doll (1954) first presented the multistage model of carcinogenesis. Crump and Howe (1984) recently extended it to the case of time-dependent dosing. Kodell et al (1987) derived bounds on the relative effectiveness of dosing at different times. Several authors extended the multistage model to incorporate cell proliferation; Moolgavkar and Venzon (1979) and Moolgavkar and Knudson (1981) presented a recent mathematical formulation of such a

model. Early versions² of some of the results in Chapter 6 were published in Krewski et al (1987).

Chapter 2.

LINEAR COMPARTMENTAL MODELS

1. Introduction

Linear compartmental models satisfy the differential equation

$$\dot{x}(t) = Rx(t) + d(t) \quad (2.1.1)$$

where $x(t)$ (n by 1) is called the state vector of the system, R (n by n) is called the transition matrix, and $d(t)$ (n by 1) is called the driving, impulse, or dosing function. In typical applications, x is the vector of quantities of some substances in n compartments, R describes the rates of flow between compartments, and d is the vector of rates of external input to each compartment.

Often, d is known, and measurements of $y(t)=x(t)+e(t)$, where $e(t)$ is a random error process, can be made. The statistical problems are to estimate R and to predict $y(t)$ under different or hypothetical experimental conditions.

For example, consider a compartmental model of the pharmacokinetics of a substance in the body. Let $x_i(t)$ be

the amount of the substance in compartment i at time t , for $i = 1, \dots, n$. Assume that the system is closed (outputs may be accommodated by including a "sink", a compartment which accumulates all inputs) and that there is no input to the system after time $t=0$.

First order linear kinetics are then described by the differential equations

$$\dot{x}_i(t) = \sum_{j \neq i} k_{ji} x_j(t) - x_i(t) \sum_{j \neq i} k_{ij} \quad (2.1.2)$$

where $k_{ij} \geq 0$ is the rate constant for flow from compartment i to j . Letting $R_{ij} = k_{ji}$ for $i \neq j$ and $R_{ii} = -\sum_{j \neq i} k_{ij}$, we write (2.1.2) in matrix form as (2.1.1) with $d(t) = 0$.

In this chapter, we derive the solution to (2.1.1) for general matrices R . Section 2 solves the homogeneous equation $\dot{x}(t) = Rx(t)$ and derives the form of the component solutions $x_i(t)$. Section 3 generalizes this to the case of constant non-zero $d(t)$. In section 4, the full solution to (2.1.1) is derived. Section 5 applies the solution to derive a simple formula relating the applied dose and the integral of the solution.

2. Solution to the Homogeneous Equation

With no input, (2.1.1) becomes the homogeneous system of differential equations

$$\dot{x}(t) = Rx(t). \quad (2.2.1)$$

This case is important for two reasons. First, it has practical application to pharmacokinetic experiments in which dosing all occurs before the start of observation, as in single intravenous or oral doses. Second, its solution, forms the basis for the solution of all other cases.

Proposition 2.2.1

The homogeneous linear compartmental system (2.2.1) with initial value $x(0)$ has the unique solution

$$x(t) = \text{Exp}(Rt)x(0) \quad (2.2.2)$$

for $t \geq 0$. Here $\text{Exp}(\cdot)$ is the matrix exponential function defined on a square matrix A by

$$\text{Exp}(A) = \sum_{i=0}^{\infty} A^i / i!. \quad (2.2.3)$$

Proof

Because the right hand side of (2.2.1) is continuous as a function of x and t and continuously differentiable with respect to all components of x , Picard's theorem (Simmons, 1972, p. 266) shows that it has a unique solution. Thus, it is only necessary to verify that (2.2.2) is that solution.

Using the convention that the derivative of a vector function with respect to a scalar is the vector of component derivatives, we have

$$\begin{aligned} \frac{d\text{Exp}(Rt)x(0)}{dt} &= \sum_{i=1}^{\infty} \frac{t^{i-1}}{(i-1)!} R^i x(0) \\ &= R \text{Exp}(Rt)x(0) \\ &= Rx(t) \end{aligned} \tag{2.2.4}$$

as required. This completes the proof. $\square$

Evaluation of the matrix exponential function using the formula (2.2.3) would be very difficult in practice, but fortunately it is not necessary. The remainder of this section describes a simpler way to calculate the matrix exponential and develops notation for use in the next section.

Let $T^{-1}JT = R$ be a Jordan decomposition of R (Lang, 1971, pp. 297-301). That is, T is invertible and

$$J = \text{diag}(J_1, \dots, J_r) \tag{2.2.5}$$

is the Jordan normal form of R , where

$$J_i = \begin{pmatrix} \rho_i & 1 & 0 & \dots & 0 \\ 0 & \rho_i & 1 & \dots & 0 \\ \vdots & \vdots & \vdots & \dots & \vdots \\ 0 & 0 & 0 & \dots & \rho_i \end{pmatrix}. \tag{2.2.6}$$

Here each J_i is s_i by s_i , with $\sum s_i = n$ and $\rho_i \in \mathbb{C}$, $i=1, \dots, r$ are the eigenvalues of R . (The ρ_i terms are not necessarily distinct. If we denote the distinct eigenvalues by λ_i , $i=1, \dots, p$, each of multiplicity m_i , then the sum of s_j for j with $\rho_j = \lambda_i$ is just m_i . In Proposition 2.3.1 below we will assume the ρ_i are ordered so that the zero eigenvalues $\rho_1 = \dots = \rho_s = 0$ come first.)

Clearly,

$$\begin{aligned} \text{Exp}(Rt) &= T^{-1} \text{Exp}(Jt) T \\ &= T^{-1} \text{diag}[\text{Exp}(J_1 t), \dots, \text{Exp}(J_r t)] T. \end{aligned} \quad (2.2.7)$$

Thus we need only calculate the exponential of a matrix of the form $J_i t$. It is easily shown by induction on n that the matrix J_i^n is of the form

$$J_i^n = \begin{pmatrix} \rho_i^n & (n-1)\rho_i^{n-1} & \dots \\ 0 & \rho_i^n & \dots \\ . & . & \dots \end{pmatrix}, \quad (2.2.8)$$

where each entry that is $k = 0, 1, \dots, s_i - 1$ steps above the diagonal is the corresponding term from the binomial expansion of $(\rho_i + 1)^n$. Thus

$$\text{Exp}(J_i t) = \sum_{n=0}^{\infty} \frac{J_i^n t^n}{n!} \quad (2.2.9)$$

will have a similar "banded" form. Here terms k steps above

the diagonal will have values the same as

$$\begin{aligned}
 (\text{Exp}(J_i t))_{1,1+k} &= \sum_{n=0}^{\infty} \frac{\binom{n}{n-k} \rho_i^{n-k} t^n}{n!} \\
 &= \frac{t^k}{k!} \sum_{m=0}^{\infty} \frac{\rho_i^m t^m}{m!} \\
 &= \frac{t^k}{k!} \exp(\rho_i t).
 \end{aligned}
 \tag{2.2.10}$$

Thus

$$\text{Exp}(J_i t) = \text{Exp}(\rho_i t) \begin{pmatrix} 1 & t & \frac{t^2}{2!} & \cdots & \frac{t^{s_i-1}}{(s_i-1)!} \\ 0 & 1 & t & \cdots & \frac{t^{s_i-2}}{(s_i-2)!} \\ \cdot & \cdot & \cdot & \cdots & \cdot \\ 0 & 0 & 0 & \cdots & 1 \end{pmatrix}.
 \tag{2.2.11}$$

We employ this representation in the following proposition.

Proposition 2.2.2

Let $\lambda_1, \dots, \lambda_p$ be the distinct eigenvalues of the transition matrix R . Then both the matrix $\text{Exp}(Rt)$ and the solution vector $x(t)$ to the homogeneous system (2.2.1) will have entries which are linear combinations of terms of the form

$$t^j \exp(\lambda_i t)
 \tag{2.2.12}$$

where $i=1, \dots, p$ and $j=0, \dots, m_i-1$.

Note

A slightly stronger result (which we do not need below) is available since j need only range up to the largest $s_k - 1$ such that $\rho_k = \lambda_1$. In cases where there are repetitions of eigenvalues among the ρ_i , this will be lower than m_1 .

Proof

The stronger result follows directly from the representations (2.2.2), (2.2.7), and (2.2.11). $\square$

3. Non-zero Constant Input

If the dosing function $d(t)$ is constant over time, then equation (2.1.1) can be written as

$$\dot{x}(t) = Rx(t) + d \quad (2.3.1)$$

where d is an n dimensional vector. This form of the model corresponds to dosing by constant infusion, with component d_i being the infusion rate into compartment i .

Proposition 2.3.1

The general solution to the constant dosing linear compartmental model (2.3.1) is

$$x(t) = \text{Exp}(Rt)[x(0) + R^{-1}d] + (R^{-1}t - R^{-1})d \quad (2.3.2)$$

where

$$R^{-1} = T^{-1} \text{diag}(I_{m_1}, 0_{s_{s+1}}, \dots, 0_{s_r}) T, \quad (2.3.3)$$

and

$$R^{-} = T^{-1} \text{diag}(0_{m_1}, J_{s+1}^{-1}, \dots, J_r^{-1}) T. \quad (2.3.4)$$

Here, I_k and 0_k are the k by k identity and zero matrices respectively. The terms T , J_i and s_i are defined in the previous section. We have here assumed for convenience that the first z eigenvalues ρ_1 to ρ_z of R are zero and correspond to the first distinct eigenvalue λ_1 , so that $\sum_{i=1}^z s_i = m_1$.

Note

If the compartmental system is closed, as in (2.1.2), R will be of less than full rank and one of the eigenvalues will necessarily be zero. If R is of full rank we adopt the convention that $m_1=0$ so that $\rho_1=\lambda_1=0$ is an eigenvalue of multiplicity zero.

Proof

Equation (2.3.1) is a nonhomogeneous linear differential equation. Its general solution is given by the sum of the general solution to the homogeneous form (2.2.1) and any particular solution to (2.3.1). Thus we need only confirm that (2.3.2) meets the initial conditions and that

$$x_p(t) = (R^{-1}t - R^{-})d, \quad (2.3.5)$$

satisfies (2.3.1).

Evaluating (2.3.2) at $t=0$ immediately confirms that it simplifies to $x(0)$ and meets the initial conditions.

Substituting (2.3.5) into (2.3.1) yields

$$R^{\perp}d = R(R^{\perp}t - R^{\perp})d + d. \quad (2.3.6)$$

Since t is arbitrary sufficient conditions are thus that both

$$R^{\perp} = -RR^{\perp} + I \quad (2.3.7)$$

and

$$0 = RR^{\perp}. \quad (2.3.8)$$

If we regard d as arbitrary as well, then these conditions are also necessary. Straight substitution of definitions (2.3.3) and (2.3.4) into (2.3.7) and (2.3.8) verifies that they meet these conditions. $\square$

We may now make a generalization to Prop. 2.2.2:

Proposition 2.3.2

The solution vector $x(t)$ to the constant input linear compartmental system (2.3.1) will have entries which are linear combinations of the terms (2.2.12) with λ_1 to λ_p and m_2 to m_p as in Proposition 2.2.2, and m_1 set to either the multiplicity of the zero eigenvalue λ_1 , or 2, whichever is larger.

Proof

We represent the first term in (2.3.2) using the result of Proposition 2.2.2. The term $(R^T t - R)d$ may then be represented in the form (2.2.12) with $\lambda_1 = 0$ and $j = 0$ and 1.

□

4. General Input

In this section we derive the solution to equation (2.1.1) for fairly general input functions. Unfortunately, without assumptions about $d(t)$ such as those in sections 2 and 3, simplifications of the solution are not available.

Proposition 2.4.1

Let $d(t)$ be piecewise continuous and bounded. Then the solution to the general linear compartmental system (2.1.1) is

$$x(t) = \text{Exp}(Rt)x(0) + \int_0^t \text{Exp}[R(t-u)]d(u)du. \quad (2.4.1)$$

Proof

Picard's theorem and the assumption that $d(t)$ is piecewise continuous and bounded guarantees the existence and uniqueness of a solution to (2.1.1) (Simmons, 1972, p.

266). Thus the demonstration that (2.4.1) is the solution is again simply a verification.

Evaluating (2.4.1) at $t=0$ gives the required result of $x(0)$. To differentiate it with respect to t , we need to differentiate the matrix exponential function. From its definition (2.2.3), it is clear that

$$\frac{d\text{Exp}(Rt)}{dt} = R\text{Exp}(Rt) = \text{Exp}(Rt)R. \quad (2.4.2)$$

Thus differentiating (2.4.1) gives

$$\dot{x}(t) = R\text{Exp}(Rt)x(0) + \text{Exp}(0_n)d(t) + \int_0^t R\text{Exp}[R(t-u)]d(u)du \quad (2.4.3)$$

where we have used the boundedness of $d(u)$ to bring the differentiation under the integral sign. Simplifying (2.4.3) gives

$$\dot{x}(t) = R\left\{\text{Exp}(Rt)x(0) + \int_0^t \text{Exp}[R(t-u)]d(u)du\right\} + d(t) \quad (2.4.4)$$

as required. $\square$

5. Area under the concentration-time curve

Several models of carcinogenic risks due to exposure to toxins postulate that the hazard is proportional to the area under the concentration-time curve, called the AUC or systemic availability (Gibaldi and Perrier, 1975), in the affected compartment. (Chapter 6 describes two models that can exhibit this property.) For this reason, the AUC has

special significance in pharmacokinetic modelling.

Proposition 2.5.1

Define

$$AUC_i = \int_0^{\infty} x_i(t) dt. \quad (2.5.1)$$

and let $d(t)$ be piecewise continuous and bounded. Then the AUC has the particularly simple form

$$AUC = -R \int_0^{\infty} d(t) dt \quad (2.5.2)$$

when the initial concentration $x(0)$ is zero and all eigenvalues of R are non-positive. If under constant dosing at the rate d compartment i of the system converges to a steady state $x^{\infty}(d)_i$, then

$$AUC_i = \int_0^{\infty} x^{\infty}[d(t)]_i dt \quad (2.5.3)$$

Notes

We would expect $x(0)$ to be zero in a pharmacokinetic model of a substance that is not naturally occurring. Hearon (1963) has shown that the eigenvalues will be non-positive provided the system conserves mass; this is discussed further in Chapter 3.

Formula (2.5.2) is especially useful when dosing is always received via a single route (so that the vector $d(t)$ changes in magnitude but not direction) since it shows that in this case the AUC is directly proportional to the area under the dose versus time curve and allows the constant of

proportionality to be estimated experimentally.

Proof

We start with the simple case of dosing at a constant rate d from time $t=0$ to $t=t_0$, after which dosing ceases. The solution for $x(t)$ is obtained from (2.3.2) for t in the interval $[0, t_0]$. For $t > t_0$, it is obtained by shifting the solution (2.2.2) so that it starts at time t_0 with value $x(t_0)$ calculated from (2.3.2), giving

$$x(t) = \begin{cases} \text{Exp}(Rt)R^{-1}d + (R^{-1}t - R^{-1})d & t \leq t_0 \\ \text{Exp}(Rt)R^{-1}d + t_0R^{-1}d - \text{Exp}[R(t-t_0)]R^{-1}d & t > t_0 \end{cases} \quad (2.5.4)$$

One problem arises when trying to calculate the area under the curve in the general model: the integral in (2.5.1) does not necessarily converge. To accommodate this, we therefore restrict it to a finite interval 0 to $M > t_0$, and denote the restricted AUC as AUC^M . When appropriate we will calculate the AUC as the limit as $M \rightarrow \infty$.

Using (2.5.4),

$$\begin{aligned} \text{AUC}^M &= \int_0^M \text{Exp}(Rt)R^{-1}d \, dt + \int_0^{t_0} tR^{-1}d \, dt + \int_{t_0}^M t_0R^{-1}d \, dt \\ &\quad - \left\{ \int_0^{t_0} R^{-1}d \, dt + \int_{t_0}^M \text{Exp}[R(t-t_0)]R^{-1}d \, dt \right\} \\ &= \int_{M-t_0}^M \text{Exp}(Rt)R^{-1}d \, dt + t_0(M-t_0/2)R^{-1}d - t_0R^{-1}d \quad (2.5.5) \end{aligned}$$

By assumption, the eigenvalues of R are non-positive. By our construction (2.3.4), R^{-1} annihilates the eigenspace of

the zero eigenvalue. Thus the components of $\text{Exp}(Rt)R^+$ all tend to zero as t becomes large, and the first term in (2.5.5) vanishes as $M \rightarrow \infty$.

We now regard (2.5.5) as n separate equations for the components of AUC^M . Since the first term vanishes and all other terms are fixed, it is clearly the behaviour of MR^+d that determines the convergence/divergence of each component. If $(R^+d)_i$ is zero, component i will converge, whereas if it is non-zero it will diverge. Thus we have, for any compartment i for which the AUC exists,

$$AUC_i = t_0(-R^+d)_i. \quad (2.5.6)$$

Recall that under continuous dosing at constant dose d , with initial $x(0)=0$, (2.3.2) gave $x(t)=[\text{Exp}(Rt)R^++R^+t-R^-]d$. By the argument above we see three things as $t \rightarrow \infty$. First, the exponential term vanishes; second, $x_i(t)$ for i with $(R^+d)_i \neq 0$ diverges; and third, $x_i(t)$ for i with $(R^+d)_i = 0$ tends to the limit $-R^-d$. Thus, if we define x^∞ to be the vector of limit or steady-state values of $x(t)$ under constant dosing at rate d , with the understanding that some of its components may be infinite, then under dosing which stops at time t_0 ,

$$AUC = t_0 x^\infty. \quad (2.5.7)$$

To generalize this result to an arbitrary dosing function $d(t)$, we consider the compartmental system as a mapping of the pair $(x(0), d(\cdot))$ onto the function $x(\cdot)$. It is clearly a linear mapping. It is also time-homogeneous, in the sense that if $x(\cdot)$ is the image of $(x(0), d(\cdot))$ and $y(\cdot)$ is the image under a time shift of $r > 0$, i.e. the image of $(x(r), \delta(\cdot))$ where $\delta(t) = d(t+r)$, then $y(t) = x(t+r)$ for all t .

If $d(t)$ is an arbitrary Riemann integrable dosing function, it can be approximated arbitrarily well (in the L_1 sense) by a sum of step functions. By the linearity of the mapping, the AUC will be the sum of areas due to each step of the step function. Since the system is time homogeneous, these can be shifted back to starting time 0 and the calculation (2.5.5) will apply. Provided $(R^{-1}d(t))_i = 0$ for all t , AUC_i for the sum of step functions is then just a sum of terms of the form (2.5.6). Since this is a bounded linear functional, it can be extended continuously to $d(t)$, and we have (2.5.2). Similarly (2.5.7) extends to (2.5.3). $\square$

Chapter 3.

A NEW PARAMETRIZATION OF LINEAR COMPARTMENTAL MODELS

1. Introduction

In the previous chapter, the general solution to the linear compartmental model (2.1.1) was derived. This chapter presents a new parametrization for the solution to the constant dosing equation

$$\dot{x}(t) = R x(t) + d. \quad (3.1.1)$$

As before let the eigenvalues of R be λ_i , each of multiplicity m_i for $i = 1, \dots, p$. The eigenvalues may be either real or complex; if complex they occur in conjugate pairs of like multiplicity. Hearon (1953, 1963) has shown that for systems in which mass is conserved (i.e. $\sum_i R_{ij} = 0$ for all j), non-negativity of the rate constants R_{ij} for $i \neq j$ implies that all eigenvalues have negative real parts except for at most one, say λ_1 , that will equal zero. He also showed that in systems obeying a chemical law known as the principle of detail balancing (Hearon, 1953), and in systems with no loops of three or more interconnecting compartments (Hearon, 1963), all eigenvalues must be real. Subsequent work has largely

concentrated on these cases, with some exceptions (e.g. Walter, 1985).

Statistical estimation of compartmental models has taken two approaches. One, in which description of the model is the aim, estimates the parameters k_{ij} . This chapter considers the other approach, that of predicting observations $y(t)=x(t)+e(t)$, where $e(t)$ is a random error process, in future or hypothetical measurements. Here the usual method has been to calculate $x(t)$ as a function of estimates of the eigenvalues and the coefficients of the linear combinations of the terms $t \exp(\lambda t)$ from Prop. 2.3.2. This approach has been called "black-box" modelling by Brown (1980).

In most applications of compartmental models in the pharmacokinetics literature it has been assumed that the eigenvalues of R are real and distinct, for several reasons. First, Hearon's (1963) work showed the eigenvalues to be real for most simple configurations of compartments such as the catenary and the mammillary*. Second, multiple

*Here, "configuration" denotes the graph-theoretic form of the model formed by considering the compartments as vertices of a directed graph with an edge from compartment i to j if and only if $R_{ij} > 0$. A catenary model is one in which the compartments form a chain, while in a mammillary model they form a star.

eigenvalues, corresponding to "resonances" in the compartmental system (Wang, 1977), can be arbitrarily well approximated on any finite time interval by distinct roots. For example,

$$(A+Bt)\exp(\lambda t) = (A-B/\epsilon)\exp(\lambda t) + (B/\epsilon)\exp[(\lambda+\epsilon)t] + o(1) \quad (3.1.2)$$

for small ϵ , where $o(1)$ represents terms tending to zero as ϵ does. Under the assumption of distinct real eigenvalues the solution to (3.1.1) becomes

$$x_j(t) = x_j^* + \sum_{i=2}^n A_{ij}\exp(\lambda_i t) \quad (3.1.3)$$

where x_j^* represents the terminal or background concentration in compartment j and contains the term corresponding to $\lambda_1 = 0$.

As a final justification for this assumption, we note that standard statistical model-building methods require a nested set of models. Expression (3.1.3) serves this purpose in situations where the number of compartments n is an unknown. It does not seem possible to write the general solution in a nested form.

The next section presents a new parametrization useful mainly in the prediction problem, and section 3 examines its properties. Section 4 presents two hypothesis tests possible in the new parametrization. Section 5 works through a simple example involving real data, and the last

section presents some general conclusions.

2. Proposed Parametrization

The need for a new parametrization is two-fold. First, there has apparently been no statistical test proposed of the hypothesis that a compartmental system under study falls in the class of those with real eigenvalues, and there is often limited a priori evidence for this. Second, degeneracies of the model, such as illustrated in the right hand side of (3.1.2) where large changes in B/ϵ can be offset by small changes in $\lambda + \epsilon$ to yield essentially the same predictions, can make estimates of the parameters of (3.1.3) numerically unstable (Wang, 1977).

The proposed parametrization is motivated by consideration of an extension of the two compartment model to general matrices R . Here, three cases are possible: the eigenvalues may be real and distinct, real and equal, or a complex conjugate pair. The second case is intermediate between the other two, being the limit either as the real roots move together or as the imaginary part of the complex roots tends to zero.

The two eigenvalues λ_1 and λ_2 (not necessarily

distinct) may thus be parametrized by two real values: the first being their mean $\mu = (\lambda_1 + \lambda_2)/2$, and the second the square of the difference from the mean to either eigenvalue $w = (\lambda_1 - \lambda_2)^2/4$. Thus the pair λ_1 and λ_2 is represented by

$$\begin{aligned} &\mu \pm \sqrt{w} && \text{if } w > 0; \\ &\mu \text{ with multiplicity 2} && \text{if } w = 0; \text{ and} \\ &\mu \pm i\sqrt{-w} && \text{if } w < 0. \end{aligned} \tag{3.2.1}$$

The choice of functions to replace the $\exp(\lambda t)$ terms in (2.2.12) is motivated by the cases of non-positive w . If $w = 0$, linear multiples of the terms $\exp(\mu t)$ and $t \exp(\mu t)$ from (2.2.12) must be representable in terms of these functions. If $w < 0$, multiples of $\cos(t\sqrt{-w})\exp(\mu t)$ and $\sin(t\sqrt{-w})\exp(\mu t)$ are required. Further, in order to satisfy typical regularity conditions of estimation and test procedures, it is necessary that the predictions be jointly continuous functions of μ , w , and the linear coefficients. (The model (3.1.1) will force them also to be continuous in t .)

Since

$$1 = \lim_{w \rightarrow 0^-} \cos(t\sqrt{-w}) = \lim_{w \rightarrow 0^+} \cosh(t\sqrt{w})$$

and

$$t = \lim_{w \rightarrow 0^-} \sin(t\sqrt{-w})/\sqrt{-w} = \lim_{w \rightarrow 0^+} \sinh(t\sqrt{w})/\sqrt{w},$$

the functions

$$f_1(t, \mu, w) = \begin{cases} \cos(t\sqrt{-w}) \exp(\mu t) & \text{for } w < 0 \\ \exp(\mu t) & \text{for } w = 0 \\ \cosh(t\sqrt{w}) \exp(\mu t) & \text{for } w > 0 \end{cases} \quad (3.2.2a)$$

and

$$f_2(t, \mu, w) = \begin{cases} \sin(t\sqrt{-w}) \exp(\mu t) / \sqrt{-w} & \text{for } w < 0 \\ t \exp(\mu t) & \text{for } w = 0 \\ \sinh(t\sqrt{w}) \exp(\mu t) / \sqrt{w} & \text{for } w > 0 \end{cases} \quad (3.2.2b)$$

satisfy the continuity requirements. Indeed, since they may be jointly represented by the series expansions

$$f_1(t, \mu, w) = \exp(\mu t) \sum_{i=0}^{\infty} \frac{w^i t^{2i}}{(2i)!} \quad (3.2.2c)$$

and

$$f_2(t, \mu, w) = \exp(\mu t) \sum_{i=0}^{\infty} \frac{w^i t^{2i+1}}{(2i+1)!} \quad (3.2.2d)$$

it is immediately clear that derivatives in μ , w , and t of all orders will exist for all real values of those parameters.

The best way to extend this parametrization to general n compartment models is not clear. Provided all real eigenvalues have multiplicity of at most two, and complex eigenvalues multiplicity of at most one, all possible solutions to (3.1.1) are representable as linear combinations of terms (3.2.2a) and (3.2.2b), although not uniquely. The non-uniqueness arises when there are more than two real eigenvalues, since they can be paired arbitrarily.

Further, the same argument that led to the approximation (3.1.2) shows that any desired multiplicities can be approximated by a model restricted in this way, albeit with the same consequent numerical difficulties.

For simplicity, therefore, we assume this restriction and propose the model for even n as

$$x_j(t) = x_j^* + \sum_{i=1}^{n/2} \{ B_{1ij} f_1(t, \mu_i, \omega_i) + B_{2ij} f_2(t, \mu_i, \omega_i) \} \quad (3.2.3a)$$

and for odd n as

$$x_j(t) = x_j^* + B_{0j} \exp(\mu_{(n+1)/2} t) + \sum_{i=1}^{(n-1)/2} \{ B_{1ij} f_1(t, \mu_i, \omega_i) + B_{2ij} f_2(t, \mu_i, \omega_i) \}. \quad (3.2.3b)$$

3. Properties of the New Parametrization

The new parametrization (3.2.3) maintains two desirable properties of the sum of exponentials form (3.1.3). First, as the number of compartments n increases, the model is extended simply by adding new terms, giving a clear hierarchical ordering of models of increasing complexity. Second, it remains a partially linear model, i.e. a sum of nonlinear terms with linear coefficients, which imparts a considerable computational advantage over a wholly nonlinear

model with the same number of parameters.

There are two main differences between the two forms. First, (3.2.3) covers the space of solutions to (3.1.1) for more general matrices R , in that under the parametrization (3.1.3) the function $x(t)$ is strictly bounded away from solutions to (3.1.1) in which R has complex eigenvalues, whereas (3.2.3) includes some of these and approximates all of them. A simple example for $n=2$ is the case

$$R = \begin{pmatrix} 0 & 1 \\ -1 & 0 \end{pmatrix}. \quad (3.3.1)$$

Here $\mu=0$ and $w=-1$ in (3.2.2), so $f_1(t,0,-1)=\cos(t)$. If $B_{111}=1$ and $B_{211}=0$ in (3.2.3), $x_1(t)$ will oscillate between $+1$ and -1 as t increases. No finite linear combination of exponentials with real exponents can exhibit this property.

In view of the results of Hearon, however, a more practically relevant difference between the models is their behaviour when all eigenvalues are real. To make this comparison, we use the measure of maximum parameter effects curvature Γ^T of Bates and Watts (1980). The curvature of a non-linear model is largely responsible for the inaccuracy of confidence limits based upon the information matrix at the maximum likelihood estimate. Bates and Watts decomposed this curvature into parameter effects and intrinsic

components. The intrinsic component of curvature measures the departure from planarity (or its higher dimensional equivalent) of the surface of predictions in the sample space mapped from all possible parameter values, and is independent of the parametrization. The parameter effects curvature measures the departure of the contours of equal parameter values on this surface from a regular grid of parallel equispaced straight lines, and can theoretically be eliminated by the correct choice of parameters. Determining the correct parameters is, however, generally an intractable problem.

Consider a simple case, $n=2$ with two real eigenvalues. (For this illustration we relax the preservation of mass condition and allow an arbitrary pair of eigenvalues, such as might be embedded in a larger model.) The intrinsic curvature of the two forms (3.1.3) and (3.2.3) is identical, since they produce the same predictions when the eigenvalues are real. However, the maximum parameter effects curvature Γ^T will differ.

Although a closed form expression for Γ^T is unknown, Bates and Watts (1980) presented an algorithm for calculating it for any particular model and experimental design. We have carried out these calculations for a simple

eight point design with observations on a single compartment under a wide variety of parameter values. A complete description of the models is given in the Appendix to this chapter.

In general, the proposed parametrization (3.2.3) had less curvature Γ^T than did the sum of exponentials model (3.1.3) when w was small (i.e. the eigenvalues were closely spaced), and also when the initial value $x_1(0)$ in the compartment under observation was small, as would be the case if blood concentrations were measured following oral or intramuscular dosing, or if tissue concentrations were measured following intravenous dosing. It had larger curvature when a single eigenvalue was dominant (i.e. the linear coefficients of the exponential terms in (3.1.3) differed greatly in magnitude), and when w was large. Further details are given in the Appendix.

4. Hypothesis tests

Two new hypothesis tests are available using the parametrization (3.2.3). As with (3.1.3), a test of model sufficiency may be made by comparing the goodness of fit with n terms to that with $n+1$. However, since (3.1.3) is

equivalent to (3.2.3) under the restriction $\omega_i > 0$, we may expect the tests based on (3.1.3) to behave as though the parameter space is restricted. In particular, the usual F ratios may not be well approximated by the F distribution. For example, a saturated model in which an exact fit to the data is obtained at each distinct time point may require more parameters than time points.

Another test motivated by this model is a test of the assumption that all eigenvalues are real and distinct versus the alternative (3.2.3). Here the likelihood ratio test (Cox & Hinkley, 1974, p. 331) would compare the likelihood of the data under the restriction $\omega_i > 0$ for all i with the unrestricted likelihood. An equivalent calculation would compare the likelihood under (3.1.3) with that under (3.2.3), keeping n fixed. Since the alternatives being compared are compound, the usual chi-square approximation to the distribution of the log of the likelihood ratio would yield conservative (high) significance levels (Cox & Hinkley, 1974, p. 332).

5. Example

In this section we give an example of fitting a compartmental model to data in the old and new

parametrizations.

The experiment being considered involved 5 animals dosed at time $t=0$ with a fixed amount of a chemical. At 24 time points ranging from 2 minutes to 21 days post-dosing, blood samples were taken and analysed for traces of the chemical. Table 3.5.1 (at the end of this chapter) shows the data from one animal, together with estimates of the precision of each observation in the form of standard deviations of the observations on all 5 animals.

Fits of models with up to 5 eigenvalues were attempted, and an analysis of variance table for the procedure is shown in Table 3.5.2. Here the models are numbered according to the following scheme. Models in the form (3.1.3) or (3.2.3) in which the background term is not included are denoted by "n", the number of eigenvalues being estimated. Those with n eigenvalues plus a background term (which can be considered as another eigenvalue known a priori to be zero) are denoted by "n+1/2". In this scheme the number of parameters in the model is just twice the number naming the model. In Table 3.5.2 the column labelled "Operation" describes the operation necessary to change the model on the line above into each model. "RSS" denotes the weighted residual sum of squares (weights being chosen as the inverse

of the variance estimate) and "SS" is the reduction in RSS due to the operation under consideration.

For this dataset, both parametrizations gave the same models as best fits in all cases. Because the two parametrizations produced identical fits in all cases, for this dataset there was no evidence at all for complex or multiple eigenvalues.

5. Conclusions

In the general linear compartmental model of three or more compartments there is no guarantee that all of the eigenvalues of the transition matrix will be real and distinct. If they are not, the usual sum of exponentials model (3.1.3) will be incorrect. If they are real and distinct but closely spaced, it will be poorly behaved statistically.

The proposed parametrization in the form of (3.2.3) smoothly extends (3.1.3) to cover the cases of distinct complex conjugate pairs of eigenvalues and double real eigenvalues. It maintains the desirable properties of hierarchical ordering of models and partial linearity, while reducing the parameter effects curvature for closely spaced eigenvalues. For widely spaced eigenvalues, it has greater

curvature, suggesting that both models should be fit to the data if no prior information on the spacing of the eigenvalues is available.

A likelihood ratio test of the hypothesis that all eigenvalues are real and distinct is available and is relatively easy to implement. If both models are fit to the data, calculation of the statistic requires simple comparison of the two log-likelihoods.

Appendix

The curvatures were calculated numerically for a series of hypothetical models with observations on a single compartment at times $t=1, 2, 4, 8, 16, 32, 64$, and 128 . Two groups of five series of calculations were run. In each of the series in the first group, λ_1 and λ_2 were varied independently over values giving half-lives $t_{1/2}(\lambda_1) = \ln 2/\lambda_1$ in half order-of-magnitude steps from 0.1 to 1000 time units. In each of the series in the second group, μ was varied over the same range of values, and w was varied from 10^{-7} to 10 in full order-of-magnitude steps.

In all models the parameter A_2 in the model expressed in the form (3.1.3) was fixed at 1 , as was the assumed

standard deviation of each observation. The 5 series in each group were distinguished by the value of A_1 . The values used were 0.01, the case of a single dominant exponential term, 0.1 for unbalanced terms, 1 for exactly balanced terms, -0.1 for unbalanced terms with opposite signs, and -1 for balanced terms with opposite signs. The latter series had the property that $x_j(0)=0$, while all of the others had positive starting values.

Because of the wide range of models used, the majority of the calculations (556 out of 810) failed for at least one of the models. The failures were all caused by apparent singularity of the information matrix (which is employed in the curvature measure of Bates and Watts (1980)). This was due to actual singularity in a few cases: those in which the eigenvalues were equal, which causes singularity in the model (3.1.3). In most cases however, the apparent singularity was caused by overflow or underflow of intermediate values. The calculations were carried out on an HP3000 minicomputer in double precision, with a maximum range of values of approximately 10^{-37} to 10^{37} .

The results of the calculations are shown in Tables 3.A.1 through 3.A.10. In these tables the value shown is the logarithm (base 10) of the ratio of the maximum

parameter effects curvature r^T under the standard parametrization (3.1.3) over that under the proposed parametrization (3.2.3), so that positive values indicate a reduction in curvature due to the new parametrization.

Table 3.5.1. Raw data for sample calculations using old and new parametrizations.

Time (min)	Obsn	Error	Time (hr)	Obsn	Error	Time (day)	Obsn	Error
2	26.2	1.7	1	7.0	0.4	1	0.31	0.13
5	17.8	2.9	2	3.9	0.8	2	0.12	0.03
10	16.4	2.1	3	3.8	0.5	3	0.10	0.03
20	12.4	1.6	4	2.9	0.4	4	0.06	0.01
30	10.2	0.5	5	3.7	0.3	5	0.03	0.01
40	8.9	0.7	6	2.8	0.4	6	0.02	0.01
50	7.2	0.5	7	1.8	0.2	7	0.020	0.004
			8	1.1	0.1	14	0.001	0.004
						21	0.0013	0.0002

Table 3.5.2. Analysis of Variance for model fitting example.

Model	Operation	DF	RSS	SS
0.5	Total (corrected)	23	2177.33	2177.33
1	Free background	1	303.20	1874.13
1.5	Add background	1	249.80	53.399
2	Free background	1	181.61	68.188
2.5	Add background	1	128.25	53.367
3	Free background	1	43.83	84.419
3.5	Add background	1	33.31	10.517
4	Free background	1	33.29	0.019
4.5	Add background	1	33.27	0.026

~~curvatures.~~ First group, $A_1 = 0.01$.

[illegible]

Table 3.A.2. Log ratio of maximum parameter effects
curvatures. First group $A_1 = 0.1$.

	$t_{1/2}(\lambda_1)$								
	0.1	0.3	1.0	3.2	10.0	31.6	100	316	1000
$t_{1/2}(\lambda_2)$									
0.1	.	.	.	.	.	.	.	.	.
0.3	.	.	0.6	-0.5	-0.6	-0.7	.	.	.
1.0	.	.	.	0.1	-0.2	-0.5	-0.9	-1.1	-1.1
3.2	.	.	-0.8	.	0.2	-0.1	-0.3	-0.4	-0.4
10.0	.	.	-1.7	-0.6	.	0.2	0.0	-0.0	-0.0
31.6	.	.	.	-1.5	-0.6	.	0.2	0.2	0.1
100	.	.	.	.	.	-0.4	.	.	0.4
316	.	.	.	.	-1.5	-0.7	.	.	.
1000	.	.	.	.	-1.6	-0.8	.	.	.

Table 3.A.3. Log ratio of maximum parameter effects
curvatures. First group, $A_1 = 1$.

	$t_{1/2}(\lambda_1)$								
	0.1	0.3	1.0	3.2	10.0	31.6	100	316	1000
$t_{1/2}(\lambda_2)$									
0.1	.	.	.	.	.	.	.	.	.
0.3	.	.	.	-0.7	.	.	.	.	.
1.0	.	.	.	-0.1	-0.8	-1.4	-1.9	-2.1	-2.1
3.2	.	-0.7	-0.1	.	-0.1	-0.6	-1.1	-1.3	-1.4
10.0	.	.	-0.8	-0.1	.	-0.1	-0.5	-0.7	-0.7
31.6	.	.	-1.4	-0.6	-0.1	.	-0.2	-0.3	-0.4
100	.	.	-1.9	-1.1	-0.5	-0.2	.	-0.4	-0.4
316	.	.	-2.1	-1.3	-0.7	-0.3	.	.	.
1000	.	.	-2.1	-1.4	-0.7	-0.4	-0.4	.	.

Table 3.A.4. Log ratio of maximum parameter effects
curvatures. First group, $A_1 = -0.1$.

	$t_{1/2}(\lambda_1)$								
	0.1	0.3	1.0	3.2	10.0	31.6	100	316	1000
$t_{1/2}(\lambda_2)$									
0.1	.	.	.	.	.	.	.	.	.
0.3	.	.	-0.3	-0.7	-0.8	-1.0	.	.	.
1.0	.	.	.	1.3	0.5	-0.3	-0.9	-1.1	-1.2
3.2	.	.	-0.7	.	1.1	0.4	0.3	0.0	-0.0
10.0	.	.	-1.7	-0.5	.	1.0	0.9	0.7	0.7
31.6	.	.	.	-1.4	-0.5	.	0.9	1.0	1.0
100	.	.	.	.	-1.2	-0.2	.	.	0.8
316	.	.	.	.	-1.5	-0.5	.	.	.
1000	.	.	.	.	-1.5	-0.6	.	.	.

Table 3.A.5. Log ratio of maximum parameter effects
curvatures. First group, $A_1 = -1$.

	$t_{1/2}(\lambda_1)$								
	0.1	0.3	1.0	3.2	10.0	31.6	100	316	1000
$t_{1/2}(\lambda_2)$									
0.1	.	.	.	.	.	.	.	.	.
0.3	.	.	-0.9	-1.0	.	.	.	.	.
1.0	.	-0.9	.	0.6	-0.6	-1.4	-1.9	-2.1	-2.1
3.2	.	-1.0	0.6	.	0.9	-0.3	-1.0	-1.2	-1.3
10.0	.	.	-0.6	0.9	.	1.1	0.0	-0.3	-0.4
31.6	.	.	-1.4	-0.3	1.1	.	1.8	1.3	1.1
100	.	.	-1.9	-1.0	0.0	1.8	.	3.6	3.1
316	.	.	-2.1	-1.2	-0.3	1.3	.	.	.
1000	.	.	-2.1	-1.3	-0.4	1.1	3.1	.	.

Table 3.A.7. Log ratio of maximum parameter effects
curvatures. Second group, $A_1 = 0.1$

[illegible]

Table 3.A.9. Log ratio of maximum parameter effects
curvatures. Second group, $A_1 = -0.1$.

[illegible]

Chapter 4.

MICHAELIS-MENTEN COMPARTMENTAL MODELS

1. Introduction

In the previous two chapters we have been concerned with linear compartmental models, where the rate of flow from compartment to compartment is proportional to the current amount in the source compartment. In this chapter we consider a model in which the flow rate is instead a non-linear function of the amount.

The most often used non-linear flow-rate function is the Michaelis-Menten function. It was first presented in 1913 (Michaelis & Menten, 1913). Briefly, it was derived as follows.

Consider the inversion of a sugar S into S^* by means of the enzyme E , according to the reaction

Assume that reaction 1 proceeds much faster than reaction 2, so that it is essentially at equilibrium. Then the law of

mass action* implies

$$[S][E] = k_1[SE] \quad (4.1.2)$$

where $[X]$ denotes the concentration of chemical species X .

Now assume the total free and bound enzyme is fixed; i.e.

$$[E] + [SE] = C \quad (4.1.3)$$

Then $[S](C - [SE]) = k_1[SE]$ which implies $[SE] = C[S]/(k_1 + [S])$.

Now the law of mass action implies that the velocity of reaction 2 is $k_2[SE]$, so we have

$$\frac{d[S]}{dt} = \frac{k_2 C [S]}{k_1 + [S]}, \quad (4.1.4)$$

the Michaelis-Menten equation. The standard form of the Michaelis-Menten equation is obtained by putting $V = k_2 C$,

$K = k_1$, $x_1 = [S]$ and $x_2 = [S']$:

$$v_{ij} = \frac{V x_1}{K + x_1} \quad (4.1.5)$$

(We write v_{ij} for the velocity of the flow from compartment i to compartment j .)

In this chapter, we use an alternate form, obtained by setting $k = k_2 C / k_1 = V / K$ and $a = 1 / k_1 = 1 / K$. This parametrization allows linear kinetics ($v_{ij} = kx_i$) as the special case $a = 0$:

*The law of mass action states that the rate of a reaction in one direction is proportional to the product of the concentrations of its reactants.

$$v_{ij} = \frac{kx_i}{1+ax_i} \quad (4.1.6)$$

As in the previous chapter, let $x(t)$ be a vector representing the amount of some substance in n compartments at time t under dosing function $d(t)$.

The general Michaelis-Menten system can then be written

$$\dot{x}_i(t) = \sum_{j \neq i} \sum_{m=1}^{n_{ji}} \frac{k_{jim} x_j(t)}{1 + a_{jim} x_j(t)} - \sum_{j=1}^n \sum_{m=1}^{n_{ij}} \frac{k_{ijm} x_i(t)}{1 + a_{ijm} x_i(t)} + d_i(t) \quad (4.1.8)$$

where $k_{jim} > 0$ and $a_{ijm} \geq 0$ ($j \neq i$) are the Michaelis-Menten coefficients for the m^{th} path from compartment i to compartment j , and k_{iim} and a_{iim} are those of the elimination paths from compartment i . (Note that under linear kinetics it is sufficient to have just one path between each pair of compartments, i.e. $n_{ij}=1$, since several linear paths with coefficients k_{ijm} and $a_{ijm}=0$ behave the same as one path with $k_{ij} = \sum_m k_{ijm}$, while Michaelis-Menten paths cannot in general be combined in this way.)

If dosing is maintained at a constant rate $d(t)=d$, then in some compartments a steady-state limit value may be

approached. As with linear kinetics, there may also be "sinks" which, having no exits, continually accumulate material and never reach a steady level. However, because the Michaelis-Menten function is bounded, there is a third possibility: some compartments may have steady-state limits for small values of d , but not for larger values. In these compartments at high doses the rate of input exceeds the total maximum rate of output. For the range of doses for which steady-state concentrations exist the limit value is generally a non-linear function of the dose d , and is difficult to calculate. Section 2 of this chapter presents an algorithm for its exact calculation in certain simple networks. Section 3 gives an iterative approximation for general networks.

Because of the difficulties in all calculations involving Michaelis-Menten systems, approximations by linear systems are often made. The assumption is that as long as saturation is not reached, a linear kinetic model will give a good description of the true kinetics. In Section 4 several rigorous forms of this assumption are proven.

2. Steady-state Solutions - Simple Networks

Consider the simple compartmental model shown in Figure 4.1 (at the end of this chapter). The model as a whole describes the pharmacokinetics of a hypothetical chemical in a single tissue. The two compartments in this model represent different forms of a chemical, rather than different physical locations. The toxicant compartment contains all of the chemical that is chemically unchanged, while the reactive metabolite compartment contains a form of the chemical that has been metabolically transformed in such a way that it can cause damage to the tissue. Because of the directions of the flows, it is clear that the amount of toxicant present at steady-state depends on the rates of uptake, activation, and elimination from the toxicant compartment. Because it does not depend on the amount of reactive metabolite nor the rates of detoxification and elimination from that compartment, it can be obtained by solving the simpler one compartment model obtained by deleting the reactive metabolite compartment. Once the steady-state level of toxicant has been determined, then the rate of the activation step is fixed, and the amount of reactive metabolite can be similarly obtained by solving a one compartment system.

This de-coupling of the solutions to the system will be a feature of any compartmental model that imposes a strict ordering on the compartments by the direction of the flows. To be precise, consider the relation $>$ defined on the compartments by $i > j$ if there exists m such that $k_{ijm} > 0$. It may be helpful to think of the ordering as i being upstream from j . If this relation can be extended by transitivity to a partial ordering of the compartments, then we call the model an "ordered compartmental system" and the following algorithm can be used to obtain the steady-state solution.

- 1) Choose any maximal element i . (Since there are only a finite number of compartments, there must be one.) This will be a compartment with no flows entering except through the constant dosing $d(t)=d$. Attempt to solve the algebraic equation $\dot{x}_i = 0$, which expands to

$$d_i = \sum_{j=1}^n \sum_{m=1}^{n_{ij}} \frac{k_{ijm} x_j}{1 + a_{ijm} x_j}, \quad (4.2.1)$$

for its unique positive solution x_i . (Here we have dropped the argument t , since we are looking for a time-independent solution.) The uniqueness comes from the fact that each of the Michaelis-Menten flows out of compartment i is a strictly increasing function of

x_i . There may be no solution, however, since the sum of these flows (the right hand side of [4.2.1]) will be bounded above if $a_{ijm} > 0$ for all j and $m=1, \dots, n_{ij}$. This corresponds to the two cases of the rate of input either meeting or exceeding the maximum rate of output. The steady-state behaviour in the latter case is an unbounded linear increase over time, and in the former an unbounded increase at less than a linear rate. In either case, the solution may formally be represented as positive infinity for subsequent steps of this algorithm.

- 2) Rewrite the system by deleting the compartment whose steady-state solution has just been obtained. If there are no other compartments left, the process is complete. Otherwise add flows coming from the solved compartment into the dosing vector for the remaining compartments. That is, if compartment j has just been solved, then for each remaining compartment i calculate a new dosing value by

$$d_i' = d_i + \sum_{m=1}^{n_{ji}} \frac{k_{jim} x_j}{1 + a_{jim} x_j} \quad (4.2.2)$$

If compartment j had no solution, use the limit values k_{jim}/a_{jim} in place of the ratios in (4.2.2). These values must be finite, since $a_{jim} > 0$ or there would have been a solution.

3) Repeat from step 1 if necessary.

Solving the algebraic equation in the first step of this algorithm may or may not be easy, depending on the exact configuration of the compartmental system. In some simpler cases, explicit solutions are available, as follows.

Suppose we are solving for the steady-state value in maximal compartment i in an ordered system. If $a_{ijm} = 0$ for all j and $m=1, \dots, n_{ij}$, then (4.2.1) reduces to

$$d_i = L x_i \quad (4.2.3)$$

where $L = \sum_{j,m} k_{ijm}$ is the sum of the linear exit rate coefficients. Thus the solution is $x_i = d_i/L$ provided the sum is non-empty (i.e. the compartment is not a sink).

If a single a_{ijm} is non-zero, then (4.2.1) reduces to

$$d_i = L x_i + \frac{k \cdot x_i}{1 + a x_i} \quad (4.2.4)$$

where L is now the sum over all terms except the one with non-zero saturability, and k and a are the coefficients from that term with subscripts suppressed for convenience. This equation may be rearranged to the quadratic equation

$$0 = a L x_i^2 + (L + k - d_i a) x_i - d_i \quad (4.2.5)$$

for x_i . Since all of the constants k , a , L and d_i are positive, the right hand side of this equation is a parabola opening upwards that is negative when $x_i=0$. Thus, the unique positive solution is given by

$$x_i = \frac{(d_i a - L - k) + \sqrt{(L + k - d_i a)^2 + 4 a k d_i}}{2 a L} \quad (4.2.6)$$

If two or more terms have positive saturability, then generally (4.2.1) is equivalent to a cubic or higher order equation, and numerical approximations to solutions to it are probably more useful than exact equations. One exception is the case where there are only two terms and both are saturable. In this case (4.2.1) becomes

$$d_i = \frac{k_1 x_i}{1 + a_1 x_i} + \frac{k_2 x_i}{1 + a_2 x_i} \quad (4.2.7)$$

where k_1 , a_1 , k_2 , and a_2 are the coefficients corresponding to the two saturable paths. This equation is equivalent to

$$0 = (a_1 a_2 d_i - k_1 a_2 - k_2 a_1) x_i^2 + (a_1 d_i + a_2 d_i - k_1 - k_2) x_i + d_i \quad (4.2.8)$$

Now, provided the dose does not exceed the maximal exit rate, i.e. $d_i < k_1/a_1 + k_2/a_2$, a solution will exist.

In this case the right hand side of (4.2.8) is a parabola opening downwards which is positive at $x_1=0$, so the unique positive solution is given by the quadratic formula

$$x_1 = \frac{-B - \sqrt{B^2 - 4AC}}{2A}, \quad (4.2.9)$$

where A, B and C are the coefficients of the terms which are quadratic, linear and constant in x_1 in (4.2.8).

3. Steady-state Approximations

Recall that in Proposition 2.5.1 we showed that in a linear compartmental system the steady-state concentration (in those compartments where it exists) was equal to $-Rd$. In this section we derive an iterative approximation valid for Michaelis-Menten systems that is based upon iterative application of a similar formula.

We assume that dosing is at a constant rate d . Since the Michaelis-Menten function (4.1.6) can be decomposed into a linear part and a non-linear part,

$$v_{ij} = kx_i - \frac{ax_i}{1+ax_i}kx_i \quad (4.3.1)$$

the differential equation describing the system can be similarly decomposed. Setting the rate of change to zero at steady-state, we then have

$$0 = [R - Q(x)]x + d \quad (4.3.2)$$

where

$$R_{ij} = \begin{cases} \sum_{m=1}^{n_{ji}} k_{jim} & (i \neq j) \\ -\sum_{l=1}^n \sum_{m=1}^{n_{il}} k_{ilm} & (i=j) \end{cases} \quad (4.3.3)$$

and

$$Q(x)_{ij} = \begin{cases} \sum_{m=1}^{n_{ji}} \frac{a_{jim} x_j}{1 + a_{jim} x_j} k_{jim} & (i \neq j) \\ -\sum_{l=1}^n \sum_{m=1}^{n_{il}} \frac{a_{ilm} x_i}{1 + a_{ilm} x_i} k_{ilm} & (i=j) \end{cases} \quad (4.3.4)$$

Our iterative procedure is described in the following proposition.

Proposition 4.3.1

Let $d(t) = d$ and $x^{(0)} = 0$. Given $x^{(i)}$, calculate the next estimate using the function g defined by

$$x^{(i+1)} = g(x^{(i)}) = -[R - Q(x^{(i)})]^{-1}d. \quad (4.3.5)$$

Then for sufficiently small d and invertible R this procedure converges to the steady state solution of the Michaelis-Menten system (4.1.8), i.e. to the solution of equation (4.3.2).

Proof

We will show that g is a contraction mapping for sufficiently small d . By its definition (4.3.4), $Q(x)$ is $O(\|x\|)$. Thus since R is invertible, there is some closed ball B of radius M such that $R - Q(x)$ is invertible for x in the ball. Define the matrix-valued function

$$G(x) = -[R - Q(x)]^{-1} \quad (4.3.6)$$

Then G is a matrix of continuously differentiable functions on a compact set B , and so each of its elements has bounded derivative on B . Since $g(x) = G(x)d$, $g(x)$ also has bounded derivatives, and the bound is proportional to $\|d\|$. Thus for some C

$$\|g(x) - g(y)\| \leq C \|d\| \|x - y\|. \quad (4.3.7)$$

Hence if $\|d\| < 1/2C$,

$$\|g(x) - g(y)\| \leq \frac{1}{2} \|x - y\| \quad (4.3.8)$$

for x and y in B . To complete the proof, we need to show that we could have chosen M such that g preserves this set. Then we can apply the contraction mapping theorem (Willard, 1970, p. 181) to show that the sequence $g(x^{(i)})$ converges.

Consider the case $d=0$. In this case $g(x)=0$, so we see by the argument above that g contracts any neighbourhood of the origin. Since g is a continuous function of the dose d as well as of x , there must be a neighbourhood of $d=0$ in which the original sphere of radius M is preserved. This completes the proof. $\square$

4. Low-dose Limits

As the magnitude of the vector x tends to zero, Michaelis-Menten kinetics approach linear kinetics. This can be seen in equation (4.1.8) which simplifies to a linear system at low levels of x . Thus, it is natural to conclude that at low doses, a linear approximation to the kinetics will be adequate. In this section we show rigorous forms of that statement to be true.

Because doses will vary in this development, we will explicitly include the dosing function in formulas for the amount vector, using $x(t;d(\cdot))$ for the general case and $x(t;d)$ for the case of constant dosing at rate d .

To examine the low-dose behaviour of a Michaelis-Menten system we examine the behaviour of the solution vector to (4.1.8) as the dosing function $d(\cdot)$ is rescaled down to 0. We will need as a regularity condition on $d(\cdot)$ that it remain bounded at all times; without loss of generality we assume that $\sum d_i(t) < 1$ for all t . We further assume for convenience that $x_i(0;d) = 0$ for all i *.

*The alternative of a non-zero starting value could be handled by considering the excess due to dosing, $x(t;d(\cdot)) - x(t;0)$. However, due to the non-linearity of the Michaelis-Menten system, this would not evolve according to Michaelis-Menten kinetics, so we avoid this case.

Proposition 4.4.1

Let $x(t; d(\cdot))$ be a Michaelis-Menten system obeying (4.1.8) and the assumptions in the paragraph above. Then it is low dose linear in the sense that for $0 \leq p$,

$$x(t; pd(\cdot)) = p y(t; d(\cdot)) + o(p) \quad (4.4.1)$$

where $y(t; d(\cdot))$ obeys the linearized form of the kinetics of x , i.e.

$$\dot{y}(t) = R y(t) + d(t) \quad (4.4.2)$$

for the matrix R defined in (4.3.3).

Proof

It follows from the zero starting value that $x_i(t; 0) = 0$ for all i and t . Thus for $0 \leq p$,

$$\frac{\partial x_i(t; pd(\cdot))}{\partial p} = \lim_{p \rightarrow 0} \frac{x_i(t; pd(\cdot))}{p}. \quad (4.4.3)$$

Let $y_i(t; p) = x_i(t; pd(\cdot))/p$. Then

$$\begin{aligned} \dot{y}_i(t; p) &= \frac{1}{p} \dot{x}_i(t; pd(\cdot)) \\ &= \frac{1}{p} \left\{ \sum_j \sum k_{jim} x_j - \sum_i \sum k_{ijm} x_i - \sum_j \frac{a_{jim} k_{jim} x_j^2}{1 + a_{jim} x_j} \right. \\ &\quad \left. + \sum_i \frac{a_{ijm} k_{ijm} x_i^2}{1 + a_{ijm} x_i} + pd_i(t) \right\} \\ &= \sum_j k_{jim} y_j - \sum_i k_{ijm} y_i \\ &\quad - p \left\{ \sum_j \frac{a_{jim} k_{jim} y_j^2}{1 + pa_{jim} y_j} - \sum_i \frac{a_{ijm} k_{ijm} y_i^2}{1 + pa_{ijm} y_i} \right\} + d_i(t). \quad (4.4.4) \end{aligned}$$

Now, equation (4.4.4) above is of the form of a

Michaelis-Menten system. In particular, $\dot{y}_i(t;d)$ is non-negative when $y_i(t;d)=0$, implying that $y_i(t;d) \geq 0$ for all t , and

$$\sum_i \dot{y}_i(t;d) \leq \sum_i d_i(t) < 1. \quad (4.4.5)$$

Thus $\sum_i y_i(t;p)$, and hence each $y_i(t;p)$, is bounded above by t for all times t .

Rewriting (4.4.4) as

$$\dot{y}_i(t;p) = ([R - pQ(y)]y)_i + d_i(t), \quad (4.4.6)$$

where R and Q are defined as in (4.3.3) and (4.3.4), we have

$$\begin{aligned} y_i(t;p) &= y_i(0;p) + \int_0^t [Ry(s;p)]_i ds \\ &\quad - p \int_0^t (Q[y(s;p)]y(s;p))_i ds + \int_0^t d_i(s) ds. \end{aligned} \quad (4.4.7)$$

Now, the boundedness of y implies that Qy is also uniformly bounded. Hence, as $p \rightarrow 0$, the integral involving Qy vanishes, and we see that in the low-dose limit

$$y_i(t;0) = y_i(0;0) + \int_0^t [Ry(s;0)]_i ds + \int_0^t d_i(s) ds. \quad (4.4.8)$$

We may also write this in the form

$$\dot{y}_i(t;0) = (Ry)_i + d_i(t), \quad (4.4.9)$$

demonstrating that $y(t;0)$ satisfies a linearized form of the system equations governing $x(t;pd(\cdot))$.

For those systems discussed in the previous section where steady-state solutions exist, if y is defined as $1/p$ times this solution, then (4.4.4) becomes

$$0 = Ry - p Q(y) y + d \quad (4.4.10)$$

Thus $\lim_{p \rightarrow 0} y$ satisfies

$$0 = Ry + d \quad (4.4.11)$$

the linearized steady-state equation.

In summary, we have shown that $y_i(t;0)$, the low-dose slope of $x_i(t;d(\cdot))$, obeys a linearized version of the kinetics of $x_i(t;d(\cdot))$. Since the time profile of any compartment of a linear system can never return to zero once positive, we see that the low-dose slope of $x_i(t;d)$ is finite and non-zero for all $t > 0$ (including $t = \infty$ when this makes sense), and hence $x_i(t;d)$ is low-dose linear.

□

Figure 4.1. A simple Pharmacokinetic Model for Metabolic Activation.

Chapter 5.

LOW-DOSE BEHAVIOUR OF MIXTURES

1. Introduction

Binary dose-response models specify the expected proportion $P(d)$ of subjects to respond at each dose d to which they may be exposed. The response may be any binary outcome, such as the absence/presence of a tumour at a specific site and time or the survival/death of the subject within a particular time interval. The dose may take many forms: it may be an amount of chemical to which the subject is exposed, a rate of prolonged exposure, a function relating the rate of exposure to the time on test, or even a complete description of an experimental protocol which may elicit the response. This chapter is concerned with the simplest cases, where the dose can be summarized in a single scalar value.

Human exposure to environmental carcinogens is generally at very low doses where a very small proportion of those exposed will respond. Concern arises partly because such large numbers of people are exposed that the

probability of some positive responses is appreciable. The difficulties in risk assessment come from the fact that direct measurement of small probabilities is extremely difficult. Unless more of the experimental subjects show the response under dosing than show it in an unexposed control group, only an upper limit on the probability may be estimated. Experiments with enough subjects to show elevated levels of rare responses are often too expensive to carry out.

One partial solution to this problem is to fit a parametric model $P(d;\theta)$ to the dose response function. Subjects (generally experimental animals) exposed at doses much higher than the environmental levels may allow estimation of θ , which can then be used to determine rates of response at lower doses. Unfortunately, for exactly the same reasons that direct measurement of the response probabilities is difficult, assessing the goodness of fit of the model in the low-dose region may not be possible.

Thus, the question arises as to whether there are any properties common to "reasonable" models that could be used to make inferences that are true regardless of whether or not the correct model has been chosen. One candidate property is low-dose linearity, represented by

$$P(d) = P(0) + \lambda d + o(d) \quad (5.1.1)$$

where $\lambda > 0$ is the slope of the dose-response curve $P(d)$ at low doses, and $o(d)/d \rightarrow 0$ as $d \rightarrow 0$. The appeal of low-dose linearity is two-fold. First, most biological models lead to low-dose linearity or sublinearity ($\lambda = 0$), while few lead to supralinearity ($P(d)$ growing so quickly that no finite λ exists). Thus low-dose linearity may be taken as a conservative upper bound on the true low-dose shape. Secondly, it has the mathematically appealing property of approximate additivity in excess risk $P(d) - P(0)$, allowing simple calculation of risk under multiple exposures and approximate linear interpolation between risks at high doses and at zero dose.

One reason to doubt low-dose linearity even if one accepts biological arguments in support of it is that the exposed population is heterogeneous. It is well-known that many cancers occur at different rates among different national groups. While some of this variation may be due to different exposures to unknown environmental carcinogens, it seems likely that the intrinsic susceptibility of individuals varies. Thus (5.1.1) may well apply to each individual, with the slope λ varying from individual to individual. Section 2 of this chapter examines this case: a heterogeneous population where each individual has a dose

response curve following (5.1.1), and determines sufficient conditions for the population dose-response function to be low-dose linear.

If the mechanism of carcinogenesis varies from individual to individual, it is conceivable that more than the slope of the dose-response curve will vary. Many dose-response curves can be approximated near dose 0 by a power β of dose (Krewski and Van Ryzin, 1981). In this case,

$$\frac{P(d)-P(0)}{d^\beta} = c + o(1). \quad (5.1.2)$$

Equation (5.1.1) is the special case $\beta=1$ and $c=\lambda$. Section 3 generalizes this definition of low dose shape and examines the case of a heterogeneous population wherein the shape β may vary from individual to individual, and derives limits on the shape of the population dose-response curve.

In practice there is a real restriction on mixtures that appears to yield our results automatically. Since the human population is finite, any mixture of individual dose response functions is a finite mixture, and the population dose-response curve is just the average of the curves over the finite population. This is a really a philosophical argument, and won't be discussed in detail. Suffice it to say that with individual dose-response curves being unknown, there is a continuum of potential curves, and these

are the subject of the discussion below.

2. Varying Low-dose Linearity

Consider a population in which the excess probability of response over background at dose d is given by $P(d|\theta)$, where $\theta \in \Theta$ indexes the population and is distributed according to the distribution $F(\theta)$. Then the population dose-response function will be

$$P(d) = \int P(d|\theta) dF(\theta). \quad (5.2.1)$$

The following proposition describes how individual low-dose linearity carries over to $P(d)$.

Proposition 5.2.1

Assume that each $P(d|\theta)$ is low dose linear, following (5.1.1) with low dose slope $\lambda(\theta)$.

(a) Further assume that for each individual there is an upper bound slope $f(\theta)$ such that

$$P(d|\theta) \leq d f(\theta) \quad (5.2.2)$$

for all $d > 0$ and that $E\{f(\theta)\} < \infty$. Then $P(d)$ will be low-dose linear with slope $E(\lambda(\theta))$.

(b) Assume instead that $E(\lambda(\theta)) = +\infty$, implying that the bounding function $f(\cdot)$ cannot exist. Then $P(d)$ will be low-dose supralinear.

Proof

The slope of $P(d)$ at the origin is given by

$$\lim_{d \rightarrow 0^+} \frac{P(d)}{d} = \lim_{d \rightarrow 0^+} \int \frac{P(d|\theta)}{d} dF(\theta). \quad (5.2.3)$$

Since $P(d|\theta) < df(\theta)$ and $\int df(\theta) dF(\theta) = dE\{f(\theta)\}$ converges, we have by the dominated convergence theorem

$$P'(0) = \int \lim_{d \rightarrow 0^+} \frac{P(d|\theta)}{d} dF(\theta) = \int \lambda(\theta) dF(\theta). \quad (5.2.4)$$

Thus $P(d)$ is low-dose linear with slope $E\{\lambda(\theta)\}$.

Conversely, suppose that $E\{\lambda(\theta)\} = +\infty$. From (5.2.3), for any $M > 0$ we have

$$\begin{aligned} P'(0) &\geq \lim_{d \rightarrow 0^+} \int_{\lambda(\theta) < M} \frac{P(d|\theta)}{d} dF(\lambda) \\ &= \lim_{d \rightarrow 0^+} \int_{\lambda(\theta) < M} \lambda(\theta) + o(d)/d dF(\theta) \\ &= \int_{\lambda(\theta) < M} \lambda(\theta) dF(\theta) \end{aligned} \quad (5.2.5)$$

by (5.1.1). Since M can be selected to be arbitrarily large, it follows that $P'(0) = +\infty$, and $P(d)$ is supralinear at low doses whenever $E\{\lambda(\theta)\} = +\infty$. $\square$

The proof given above depends on the bounding function $f(\theta)$ in deriving (5.2.4). It is not true that any mixture of low-dose linear curves with finite expected slope is itself low-dose linear, as the following example shows.

Let

$$P(d|M) = \begin{cases} d & \text{if } d < 1/2M \\ 1/2M + (1-1/2M)(2Md-1) & \text{if } 1/2M < d < 1/M \\ 1 & \text{if } 1/M < d \end{cases} \quad (5.2.6)$$

for $M > 1$. These functions have slope 1 up to $1/2M$, from which they proceed in a straight line to a value of 1 at $1/M$ and higher doses. They are thus continuous increasing dose-response curves with a low-dose slope of 1 for all M . If $F(M)$ is the distribution function for the value of M in the population, then since $P(d|M) \geq I(\{M: d > 1/M\})$, where I is the indicator function, the population dose response curve is bounded below by the probability that $d > 1/M$, i.e.

$$P(d) \geq 1 - F(1/d). \quad (5.2.7)$$

Thus arbitrarily quickly increasing population dose-response functions can be formed by the appropriate choice of F . In particular, letting $F(M) = 1 - \sqrt{1/M}$ for $M > 1$ yields

$$P(d) \geq \sqrt{d} \quad (5.2.8)$$

a function which is clearly supralinear.

3. Varying Low-dose Shapes

If we make the reasonable assumption that dose-response curves are non-decreasing functions, then the shape of the function giving excess risk over background is bounded above by some power of dose. Our first proposition formalizes this definition of low-dose shape.

Proposition 5.3.1

For any non-decreasing function $P(d)$ which tends to 0 as $d \rightarrow 0$ there exists a largest power $0 \leq \beta_0 \leq +\infty$ such that

$$\lim_{d \rightarrow 0^+} \frac{P(d)}{d^\beta} = 0 \quad (5.3.1)$$

if $0 < \beta < \beta_0$.

Proof

Note that the set of values of β for which (5.3.1) is true contains 0 by assumption and must be an interval, so we let β_0 be the least upper bound on this set or $+\infty$ if it is unbounded. □

We call β_0 the "low-dose shape" of the dose-response function. Krewski and Van Ryzin (1981) defined low-dose shape to be the power of dose which produces a finite non-zero limit in the left-hand side of equation (5.3.1). Such a power does not exist for all dose-response functions, but it is unique when it does exist. In these cases it will equal the definition above, since all smaller powers will produce a zero limit and all higher powers an infinite limit.

The main result of this section is that if a population

consists of a mixture of individuals with differing dose-response curves, then the low-dose shape of the population dose-response function is less than or equal to almost all of the low-dose shapes in the population. The formal statement follows.

Proposition 5.3.2

Let $\theta \in \Theta$ index the individual dose-response curves $P(d|\theta)$ in the population, and let $F(\theta)$ be the distribution of dose-response curves within the population. For each θ , let $\beta(\theta)$ be the low-dose shape of the associated dose-response curve. We assume that $\beta(\cdot)$ is a measurable function with respect to F and that $\lim_{d \rightarrow 0^+} \frac{P(d|\theta)}{d^\beta}$ exists for all β for almost all θ . Let

$$P(d) = \int P(d|\theta) dF(\theta) \quad (5.3.2)$$

be the population dose response curve, with low-dose shape β_0 . Then $\beta_0 \leq \text{ess inf } \{\beta(\theta)\}$.

Proof

For $\beta < \beta_0$

$$\begin{aligned} 0 &= \lim_{d \rightarrow 0^+} \frac{P(d)}{d^\beta} \\ &= \lim_{d \rightarrow 0^+} \int \frac{P(d|\theta)}{d^\beta} dF(\theta) \end{aligned} \quad (5.3.3)$$

Fatou's Lemma then yields

$$0 = \int \lim_{d \rightarrow 0^+} P(d|\theta)/d^\beta dF(\theta), \quad (5.3.4)$$

where lim denotes the limit inferior. But if an integral of a non-negative quantity is zero, then the integrand must be zero almost everywhere, and we have for almost all θ

$$\begin{aligned} 0 &= \lim_{d \rightarrow 0^+} \frac{P(d|\theta)}{d^\beta} \\ &= \lim_{d \rightarrow 0^+} \frac{P(d|\theta)}{d^\beta} \end{aligned} \quad (5.3.5)$$

where the limit inferior is elevated to a limit by our hypothesis that the limit exists for almost all θ . Thus for those θ for which (5.3.5) holds, $\beta(\theta) \geq \beta$. Since β may be arbitrarily close to β_0 , we also have $\beta(\theta) \geq \beta_0$. Thus

$$\begin{aligned} \beta_0 &\leq \sup \{ \beta : \Pr(\beta(\theta) < \beta) = 0 \} \\ &= \text{ess inf } \{ \beta(\theta) \}. \end{aligned} \quad (5.3.6)$$

□

The example (5.2.6) in the previous section, in which $\beta(\theta)=1$ for all individuals in the population, but supralinearity ($\beta_0=1/2$) could occur, indicates that the inequality in (5.3.6) is necessary.

Chapter 6.

CARCINOGENIC RISK ASSESSMENT

WITH TIME-DEPENDENT EXPOSURE PATTERNS

1. Introduction

The prediction of carcinogenic risks requires the use of a mathematical model to relate the frequency of occurrence of cancer to the level of exposure to a carcinogen. The previous chapter considered general properties of such dose-response models when the dosing regimen could be represented by a single value. This chapter develops a technique for use when dosing varies over time and applies it to two specific models.

The first model considered is the Crump & Howe (1984) extension to time-dependent dosing of the Armitage & Doll (1954) multistage model of carcinogenesis. The basic assumption in this model is that a cancer precursor is the last stage in a sequence of k random transitions undergone by a single cell or cell line. The instantaneous transition rate $\lambda_i(t)$ from stage i to stage $i+1$ is proportional to a linear function of the current dosing level. That is,

$$\lambda_1(t) = \alpha_1 + \beta_1 d(t) \quad (6.1.1)$$

where $\alpha_1 > 0$, $\beta_1 \geq 0$ are constants determining the background transition rate and the increase in the transition rate per unit dose respectively, and $d(t)$ is a measure of the dose at time t .

It can then be shown (Crump & Howe, 1984) that in the limit as the number of cells becomes large, the probability of a tumour by time t is approximately

$$P(t) = 1 - \exp[-H(t)] \quad (6.1.2)$$

where the cumulative hazard $H(t)$ is given by

$$H(t) = \int_0^t \int_0^{u_k} \dots \int_0^{u_2} \prod_{i=1}^k [a_i + b_i d(u_i)] du_1 \dots du_k. \quad (6.1.3)$$

In section 2 of this chapter, we consider the cumulative hazard (6.1.3) as an expectation, and derive simple formulas and bounds relating it to the dosing function $d(t)$.

A more biologically realistic model of carcinogenesis due to Moolgavkar & Venzon (1979) and Moolgavkar & Knudson (1981) is known as the birth-death-mutation model. In this model (Figure 6.1 at the end of this chapter) two transitions are necessary to form a cancer precursor. The transitions occur during cell division and occur at rates λ_1 and λ_2 . In the first transition, a mutation causes

one daughter of a normal cell to transform into an intermediate or initiated cell. Such initiated cells may then undergo malignant transformation during a subsequent cell division to give rise to a cancerous lesion. The model explicitly takes into account the rates of birth α and death β of initiated cells through the net birth rate parameter $\delta = \alpha - \beta$. The pool of normal cells is assumed to be so large that their approximate number is taken as known. Thorslund et al (1987) extended this model to accommodate time dependency by allowing λ_1 , λ_2 and δ to depend on time. They showed that in this case the cumulative hazard function is approximated by

$$H(t) = \int_0^t \int_0^{u_2} \lambda_1(u_1) \lambda_2(u_2) X(u_1) \exp[r(u_2) - r(u_1)] du_1 du_2 \quad (6.1.4)$$

where

$$r(t) = \int_0^t \delta(u) du \quad (6.1.5)$$

and $X(t)$ represents the number of normal cells at risk of initiation at time t . In section 3 of this chapter, we derive formulas relating this hazard function and the dosing function.

2. The Multistage Model

Crump & Howe (1984) derived the expression (6.1.3) for

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the cumulative hazard function for the multistage model under time dependent dosing. Based on this expression, they were able to calculate the effect of time-dependent dosing where $d(t)$ is a step function by integrating (6.1.3). A much simpler derivation of their results is possible, based on representing the multiple integral in (6.1.3) as an expectation of a function of several random variables.

There is no unique way to do this. We choose the simplest expectation representation by assuming a constant density and taking the expectation of the integrand (multiplied by a suitable normalizing constant). Doing this, we see that

$$H(t) = \frac{t^k}{k!} E \left\{ \prod_{i=1}^k [a_i + b_i d(U_{i:k})] \right\} \quad (6.2.1)$$

where $E(\cdot)$ represents expectation with respect to the distribution of $U_{1:k} < \dots < U_{k:k}$, the order statistics from k independent samples from a uniform $[0, t]$ distribution.

Single Stage Dose Dependent

The advantage of writing the cumulative hazard function in the form of (6.2.1) comes mainly from the understanding of the model that it imparts. In the case of only one dose dependent step, a particularly simple interpretation

results.

Suppose only step r is dose-dependent, i.e. $b_r > 0$ and $b_i = 0$ if $i \neq r$. Then

$$\begin{aligned} H(t) &= \frac{t^k}{k!} \left(\prod_{i=1}^k a_i \right) E \left\{ 1 + \frac{b_r}{a_r} d(U_{r,k}) \right\} \\ &= a(t) + b_r(t) d_r^*(t), \end{aligned} \quad (6.2.2)$$

where

$$a(t) = \frac{t^k}{k!} \prod_{i=1}^k a_i, \quad (6.2.3)$$

$$b_r(t) = (b_r/a_r) a(t), \quad (6.2.4)$$

and

$$d_r^*(t) = E(d(U_{r,k})). \quad (6.2.5)$$

In (6.2.2), $a(t)$ represents the background cumulative hazard at time t , while $d_r^*(t)$ is the dose which, if given constantly from time 0 to time t , would produce the same hazard as the actual dosing regimen represented by $d(\cdot)$. The expression $b_r(t)$ may thus be interpreted as the rate of accrual of cumulative hazard per unit of constant dose.

A further advantage of writing the hazard function as (6.2.1) is the fact that the properties of the uniform order statistics are well known. Thus, we may calculate (6.2.5) using

$$E(d(U_{r,k})) = \int_0^t d(u) f(u; r, k-r+1, t) du, \quad (6.2.6)$$

where $f(u;r,k-r+1,t)$ is the marginal density of the r^{th} uniform $[0,t]$ order statistic. This density is a non-standard Beta($r,k-r+1$) density, with

$$f(u;r,k-r+1,t) = t \frac{u^{r-1}(t-u)^{k-r}}{B(r,k-r+1)} \quad 0 < u < t \quad (6.2.7)$$

where $B(r,k-r+1)$ is the standard beta function with parameters r and $k-r+1$.

It follows from the simple form (6.2.2) that if the time t is taken to be fixed, the cumulative hazard is of the same general form as that of a single stage model. Thus if inferences were to be drawn from an experiment in which all subjects received constant dosing over the same period, without prior information the two models would be completely indistinguishable. If only a single stage is dose-dependent, information about the number of stages in the model can only be gleaned from experiments in which multiple time-varying exposure regimens are used, and those in which the observation times t vary.

The source of the information in experiments with time-varying exposure is the fact that under (6.2.1) the relative effectiveness of dosing varies over time. If we take constant dosing from time 0 to time t as the standard, then we can define a measure of relative effectiveness

$$R(u) = t f(u) \quad (6.2.8)$$

where $f(\cdot)$ is the density function in (6.2.7). The value $R(u)$ will then measure how effective it would be to concentrate a dose at time u as compared with spreading it uniformly over the interval $[0, t]$. Figure 6.2 graphs these functions for $1 \leq k \leq 6$ and $1 \leq r \leq k$. Examination of these graphs confirms the argument of Day and Brown (1980) that early exposure is more effective than late exposure when an early stage is dose-dependent, and late exposure is more effective when a late stage is dose-dependent.

More generally, we can define $R(d(\cdot))$ to be the relative effectiveness of the dosing regimen $d(\cdot)$ as compared to administering the same total dose at a constant rate. Then

$$R(d(\cdot)) = d^*(t)/\bar{d}(t) \quad (6.2.9)$$

where

$$\bar{d}(t) = \int_0^t d(u) du / t, \quad (6.2.10)$$

is the time-weighted average dose.

In the absence of information about the relative effectiveness function, simple averaging or amortization of a time-varying dose might be used. A consequence of the formula (6.2.7) is that this simple amortization of dose will be correct when $k=1$, since the beta distribution with

parameters 1 and 1 is the uniform distribution. In general for $k > 1$, however, it will not be accurate.

To illustrate the points above consider the case of $k=6$ with $r=1$, and suppose that dosing is constant at a rate d in the first quarter of the period $[0, t]$ (Figure 6.3). In this case the equivalent constant dose $d_1(t)$ will be 83% of d , more than three times the amortized dose of $d/4$.

Kodell et al (1986) considered the consequences of using average lifetime dose rate to predict risk from intermittent dosing, and showed that the excess risk from an intermittent dose is at most k times the risk from continuous dosing with the same total dose administered, regardless of which stage(s) are dose dependent. Writing the hazard function in the form (6.2.1) allows simple calculation of this bound when a single stage is dose dependent and improvement on it in the case where the number r of the dose dependent stage is known.

First we note that by (6.1.2) the excess risk is bounded above by the excess cumulative hazard. Thus a bound on $R(d(\cdot))$ will bound the relative excess risk. By the definition of the relative effectiveness $R(u)$, we see that its maximum value over $0 \leq u \leq t$ is the upper bound we are

seeking. Thus we have

$$R(d(\cdot)) \leq \frac{k! (r-1)^{r-1} (k-r)^{k-r}}{(r-1)! (k-r)! (k-1)^{k-1}} \quad (6.2.11)$$

$$\leq \begin{cases} k & \text{if } r=1 \text{ or } k \\ k^{3/2} (2\pi(r-1)(k-r))^{-1/2} & \text{otherwise} \end{cases} \quad (6.2.12)$$

$$\leq k, \quad (6.2.13)$$

where the bound (6.2.11) is just the mode of a standard beta density. (Here we have used the convention that $0^0=1$.) The bound (6.2.12) is based on Stirling's formula and is proven in the Appendix. The last bound (6.2.13) follows from the fact that $(r-1)(k-r) \geq (k-2)$ when $1 < r < k$.

Two or More Stages Dose-Dependent

When two stages r and s , $1 \leq r < s \leq k$, are dose dependent the situation is more complicated. In this case the hazard function will be composed of four terms instead of two. Here,

$$H(t) = a(t) + b_r(t) d_r^*(t) + b_s(t) d_s^*(t) + b_{rs}(t) d_{rs}^*(t). \quad (6.2.15)$$

where

$$b_{rs}(t) = (b_r b_s / a_r a_s) a(t) \quad (6.2.16)$$

and

$$d_{rs}^*(t) = E(d(U_{r,k}) d(U_{s,k})). \quad (6.2.17)$$

The joint distribution of the r^{th} and s^{th} order statistics from k uniform $[0, t]$ variates, necessary to evaluate the expectation in (6.2.17), is well known and may be found in Kendall and Stuart (1969, vol 1, p. 325).

At uniformly low doses $d_{r,s}(t)$ will be negligible in comparison to $d_r(t)$ and $d_s(t)$, in which case

$$H(t) \approx a(t) + b_r(t)d_r(t) + b_s(t)d_s(t). \quad (6.2.18)$$

It follows that an upper bound on $R(d(\cdot))$ at low doses is obtained by choosing the larger of the two bounds given by (6.2.11) (or 6.2.12) for stages r and s separately.

Expressions for $H(t)$ when more than two steps are dose-dependent involve expectations of all combinations of products of order statistics, and in general, will be too cumbersome to be of any practical use. When low-dose behaviour is being considered, however, products of dose terms will be negligibly small, and $H(t)$ may be approximated by

$$H(t) = a(t) + \sum_{i=1}^k b_i(t)d_i(t). \quad (6.2.19)$$

At low doses, $R(d(\cdot))$ is thus bounded above by k , the bound given by (6.2.11) when $r=1$ or $r=k$.

3. The Birth-Death-Mutation Model

Moolgavkar & Venzon (1979) and Moolgavkar and Knudson (1981) developed several birth-death-mutation models of carcinogenesis, including the one shown schematically in Figure 6.1. Thorslund et al (1987) derived the cumulative hazard function (6.1.4) of this model under the assumption that the number of normal cells $X(u)$ is essentially deterministic and that the rate of production of cancerous cells is low. In this section we explore various ways of writing this cumulative hazard function as an expectation to elucidate the properties of the birth-death-mutation model under time-dependent dosing.

As we did in section 2, we write

$$H(t) = E(\lambda_1(U_1)\lambda_2(U_2)) k_1(t). \quad (6.3.1)$$

where U_1 and U_2 , $0 < U_1 < U_2 < t$, are random variables with joint density

$$f(u_1, u_2; t) = X(u_1) \exp[\tau(u_2) - \tau(u_1)] / k_1(t), \quad (6.3.2)$$

where the normalizing constant $k_1(t)$ is calculated according to

$$k_1(t) = \int_0^t \int_0^{u_2} X(u_1) \exp[\tau(u_2) - \tau(u_1)] du_1 du_2. \quad (6.3.3)$$

If $\delta(\cdot) = 0$, and $X(\cdot)$ is constant, then (6.3.2) is constant in u_1 and u_2 and the birth-death-mutation model

reduces to two-stage model as discussed in section 2.

If we assume that $\delta(\cdot)$ is independent of dose, but $\lambda_1(t)$ and $\lambda_2(t)$ are linear functions of dose as in (6.1.1), then equation (6.2.15) holds with $r=1$ and $s=2$. Here we have

$$a(t) = a_1 a_2 k(t) \quad (6.3.4)$$

while the previous definitions (6.2.4), (6.2.5), (6.2.16) and (6.2.17) still hold, with the expectations being taken with respect to the distribution (6.3.2). Thus, as in (6.2.18), the marginal densities of U_1 and U_2 are again sufficient to characterize the low dose behaviour of the model.

In general the relative effectiveness $R(d(\cdot))$ of a variable dosing regimen $d(\cdot)$ depends on tissue growth through $X(\cdot)$. In order to illustrate the properties of the two-stage birth-death-mutation model we will first assume that $X(\cdot)$ is constant. The cases where $\delta(\cdot)$ is constant and dose-dependent are treated separately below. Two examples in which $X(\cdot)$ and $\delta(\cdot)$ are time-dependent are provided at the end of this section.

Constant Promotion

If $\delta(\cdot) = \delta \neq 0$ is constant over time, then

$$f(u_1, u_2; t) = \exp[(u_2 - u_1)\delta] / k_1(t) \quad 0 < u_1 < u_2 < t \quad (6.3.5)$$

and

$$k_1(t) = (e^{t\delta} - t\delta - 1) / \delta^2. \quad (6.3.6)$$

The marginal distributions of U_1 and U_2 with δ constant are given by

$$f_1(u_1; t) = \delta \frac{e^{(t-u_1)\delta} - 1}{e^{t\delta} - t\delta - 1} \quad 0 < u_1 < t \quad (6.3.7)$$

(Figure 6.4) and

$$f_2(u_2; t) = \delta \frac{e^{u_2\delta} - 1}{e^{t\delta} - t\delta - 1} \quad 0 < u_2 < t \quad (6.3.8)$$

respectively. As in (6.2.2), if only stage i is dose dependent then the equivalent constant dose is $d_i^*(t)$.

Several properties of these distributions are important in determining the behaviour of the birth-death-mutation model. As $\delta \rightarrow -\infty$, the marginal density $f_1(u_1; t)$ in (6.3.7) tends pointwise to the uniform density $1/t$ for $u_1 < t$ (the endpoint $u_1 = t$ remaining equal to 0 for all δ). Thus, we see that large negative values of δ lead to near uniform effectiveness of dosing as in the multistage model with $k=1$. The density tends uniformly to the triangular density $2(t-u_1)/t^2$ as $\delta \rightarrow 0$, the same density as that of the first stage of a multistage model with $k=2$. As $\delta \rightarrow +\infty$, the density tends pointwise to 0 for $u_1 > 0$,

while the endpoint u_1 tends to infinity. Thus large positive values of δ are associated with behaviour unlike that of any multistage model.

If only the first stage is dose-dependent, then the mode of the density $f_1(u_1; t)$ in (6.3.7) determines an upper bound on the relative effectiveness $R(d(\cdot))$ as discussed in the previous section. For all δ , the density is a strictly decreasing function of u_1 , so its mode occurs at $u_1=0$. Thus we have

$$R(d(\cdot)) \leq t f_1(0; t) = t\delta \frac{e^{t\delta}-1}{e^{t\delta}-t\delta-1}. \quad (6.3.9)$$

Because $tf_1(0; t)/t\delta \rightarrow 1$ as $\delta \rightarrow \infty$, we see that large positive values of δ result in relative effectiveness as large as $t\delta$ if dosing is made near time 0, somewhat similar to a multistage model with $k=t\delta$. The quantity $t\delta$ can be interpreted as the net birth rate per initiated cell over a time interval of length t . The explanation for this behaviour is clear: large values of δ correspond to a large net birth rate for initiated cells, so that initiated cells proliferate rapidly. Earlier transitions from normal cells to initiated cells thus lead to a larger pool of initiated cells than do later ones and correspondingly more opportunities for second stage mutations.

A third property of interest is that the distribution (6.3.5) is symmetric in the sense that (U_1, U_2) has the same joint distribution as $(t-U_2, t-U_1)$. Thus if only the second stage is dose-dependent, dosing at $u_2=t$ has the greatest effect, and the bound (6.3.9) remains unchanged. This large relative effectiveness with $\delta > 0$ of dosing later in life may be attributed to the larger pool of spontaneously occurring initiated cells available for malignant transformation at that time.

Dose-Dependent Promotion

The general case in which $\delta(u)$ may be dose dependent cannot be effectively handled by the technique of considering integrals as expectations, because $\delta(u)$ enters the cumulative hazard function (6.3.1) in a non-linear way. Here our results are limited to low dose limits.

Suppose $\delta(u) = \delta + b_3 d(u)$. Then

$$\begin{aligned} \exp[\tau(u_2) - \tau(u_1)] &= \exp[\delta(u_2 - u_1)] \exp[b_3 \int_{u_1}^{u_2} d(u) du] \\ &\approx \exp[\delta(u_2 - u_1)] [1 + b_3 \int_{u_1}^{u_2} d(u) du], \end{aligned} \quad (6.3.10)$$

provided $b_3 \int_{u_1}^{u_2} d(u) du$ remains small. Thus if we write v_1 , v_2 and v_3 in place of u_1 , u , and u_2 respectively, the excess cumulative hazard $\Delta H(t)$ at time t due to the effect of dosing on the net birth rate is approximately

$$\begin{aligned} \Delta H(t) &\approx b_3 \int_0^t \int_0^{v_3} \int_{v_1}^{v_3} d(v_2) \lambda_1(v_1) \lambda_2(v_3) X(v_1) \exp[\delta(v_3 - v_1)] ds dv_1 dv_3 \\ &= b_3 E\{d(V_2) \lambda_1(V_1) \lambda_2(V_3)\} k_2(t) \end{aligned} \quad (6.3.11)$$

for random variables $0 < V_1 < V_2 < V_3 < t$ with joint density

$$f(v_1, v_2, v_3; t) = X(v_1) \exp[\delta(v_3 - v_1)] / k_2(t). \quad (6.3.12)$$

Here as in (6.3.3) $k_2(t)$ is a normalizing constant chosen so that the density (6.3.12) integrates to unity. The total cumulative hazard will then be approximately

$$H(t) \approx E\{\lambda_1(U_1) \lambda_2(U_2)\} k_1(t) + b_3 E\{d(V_2) \lambda_1(V_1) \lambda_2(V_3)\} k_2(t), \quad (6.3.13)$$

where the constant promotion density (6.3.5) is used for U_1 and U_2 .

When $\lambda_1(\cdot)$ and $\lambda_2(\cdot)$ are constant, the excess hazard is determined entirely by the marginal density of V_2 . If $X(\cdot)$ is constant, then (6.3.12) may be integrated to give this density as

$$f_3(v_2; t) = \frac{2\delta \sinh(\delta v_2/2) \sinh(\delta(t - v_2)/2)}{\delta t \cosh(\delta t/2) - 2 \sinh(\delta t/2)}. \quad (6.3.14)$$

This density possesses several interesting properties. It

is symmetric in v_2 about $t/2$, as can be seen from the exchangeability of v_2 and $t-v_2$ in (6.3.14). Perhaps surprisingly, it is also symmetric in δ about 0. This follows from the fact that $\sinh(\cdot)$ is an odd function while $\cosh(\cdot)$ is even. Thus the numerator is the product of 3 odd functions, so is odd, while the denominator is the difference of two odd functions, so it is odd as well. Thus the whole expression is even in δ as claimed.

The mode of (6.3.14) occurs at $v_2=t/2$. To see this, we employ the product formula for $\sinh(\cdot)$ (Abramowitz & Stegun, p. 84) to rewrite the numerator of (6.3.14) as

$$\begin{aligned} & 2\delta \sinh(\delta s/2) \sinh(\delta(t-v_2)/2) \\ &= \delta \{ \cosh[\delta t/2] - \cosh[\delta(t/2 - v_2)] \}. \end{aligned} \quad (6.3.15)$$

Since $\cosh(x)$ achieves its minimum value of 1 at $x=0$, we see that the maximum of (6.3.15) occurs at $v_2 = t/2$. Because the denominator of the density (6.3.14) is independent of v_2 , $v_2=t/2$ is the mode, and dosing at this time will have the largest relative effect.

As can be seen in Figure 6.5, however, the relative effectiveness of dosing is bounded above by $3/2$. To prove this, we evaluate (6.3.14) at its mode $t/2$ (with the numerator in the form (6.3.15), and writing z for $\delta t/2$ and $2z/t$ for δ) as

$$f_3(t/2;t) = (1/t) \frac{z \cosh(z) - z}{z \cosh(z) - \sinh(z)} \quad (6.3.16)$$

Both the numerator and denominator of the fraction in (6.3.16) are easily expanded into Taylor series that are convergent for all z . Cancelling the common factor of z^3 , we have

$$f_3(t/2;t) = (1/t) \frac{\sum_{n=0}^{\infty} z^{2n}/(2n+2)!}{\sum_{n=0}^{\infty} (2n+2)z^{2n}/(2n+3)!} \quad (6.3.17)$$

For $z > 0$, the ratio of the n^{th} term in the series in the numerator of (6.3.17) to the corresponding term in the denominator is $(2n+3)!/[(2n+2)(2n+2)!] = (2n+3)/(2n+2)$. Since this ratio is bounded above by $3/2$ for all n , the same bound holds for the ratio of the series in (6.3.17), and an upper bound on $f_3(v_2;t)$ is $3/2t$. Taking the limit as $z \rightarrow 0$ (which implies $\delta \rightarrow 0$ for any $t > 0$) in (6.3.17) yields this upper bound. Thus the relative effectiveness of dosing at time $t/2$ is largest when $\delta = 0$.

The complete form of $f_3(v_2;t)$ in the limit as $\delta \rightarrow 0$ may be most easily calculated by integrating the limit of the joint density (6.3.12), since it remains bounded near $\delta = 0$. Straight substitution of $\delta = 0$ shows that for constant $X(\cdot)$ the density (6.3.12) is constant, whence $f_3(v_2;t) = 6v_2(t-v_2)/t^3$, which is the same density

as that of $U_{2,3}$ as given in (6.2.7).

Two or More Processes Dose Dependent

The cumulative hazard function of the two-stage birth-death-mutation model when two or three of $\lambda_1(\cdot)$, $\lambda_2(\cdot)$ and $\delta(\cdot)$ are simultaneously dose dependent cannot, in general, be simplified beyond (6.1.4). However, at low doses approximations similar to (6.2.19) but based upon (6.3.13) will hold. In this case

$$H(t) \approx a(t) + \sum_{i=1}^3 b_i(t) d_i^*(t) \quad (6.3.18)$$

where

$$b_3(t) = a_1 a_2 b_3 k_2(t) \quad (6.3.19)$$

and

$$d_3^*(t) = E(d(V_2)). \quad (6.3.20)$$

The other terms in (6.3.18) are as defined previously in this section for the case of constant promotion. Thus the relative effectiveness $R(d(\cdot))$ is bounded above by the larger of $\frac{t\delta(e^{t\delta}-1)}{(e^{t\delta}-t\delta-1)}$ and $3/2$, the former quantity being the bound (6.3.9) and the latter based on the bound on (6.3.14).

Effects of Tissue Growth

The preceding results were obtained under the simplifying assumption that the number of normal cells $X(\cdot)$ does not vary over time. However, most tissues will vary in size as the individual matures. To examine the impact of changing tissue size, we consider two examples in which $X(\cdot)$ varies over time.

In both examples, we assume that the target tissue is the human liver. The number of cells $X(\cdot)$ is modelled by assuming it to be proportional to the mean or median liver weight for males as reported in Snyder et al (1974, pp 145-6; see Figure 6.6). The marginal density functions are obtained by numerical integration of (6.3.2).

In the first example $\delta(\cdot)$ is assumed to be constant over time. The relative effectiveness of dosing when $X(\cdot)$ varies is shown in Figure 6.7. Since most of the variation in $X(\cdot)$ occurs early in life the relative effectiveness differs most from that obtained when $X(\cdot)$ is constant (Figure 6.4) when only the first stage is dose-dependent (Figure 6.7a). When the number of liver cells is small, the number of first stage mutations is reduced proportionately, thereby reducing the effectiveness of early

dosing. In contrast, when only the second stage is dose-dependent, the relative effectiveness with $X(\cdot)$ variable differs little from that when $X(\cdot)$ is constant (Figure 6.7b). Still, as in the case of constant $X(\cdot)$, the highest relative effectiveness of dosing occurs early in life when the first stage is dose-dependent and late in life when the second stage is dose-dependent.

The assumption that the net growth rate of intermediate cells $\delta(\cdot)$ is constant over time is probably unrealistic. Young individuals' organs grow in size while adults' do not, indicating that the growth rate of normal tissue is not constant. Our second example attempts to accommodate this by assuming that initiated cells proliferate at a rate proportional to the growth rate of normal cells. Specifically, we assume that $\delta(u) = CX'(u)/X(u)$, which implies that

$$r(u_2) - r(u_1) = C \log [X(u_2)/X(u_1)] \quad (6.3.21)$$

in equation (6.3.2). Figure 6.8 shows the relative effectiveness curves under this assumption. Since the growth of intermediate cells occurs primarily early in life, the growth rate has the greatest impact on the relative effectiveness of dosing when the first stage is dose-dependent, and very little when the second stage depends on dose.

Qualitatively, the impact of the variability in $X(\cdot)$ is reduced when $C \geq 1$. Here the curves resemble a mixture of those shown in Figure 6.4, for the case of constant promotion. During periods when the promotion rate exceeds the normal cell growth rate, the curves resemble constant high promotion curves. During periods when the growth rates are nearly equal (either late in life when they are both small, or early in life if $C=1$), the curves behave as do those with promotion near zero. As $C \rightarrow 0$, the curves tend to the zero promotion variable $X(\cdot)$ curve shown in Figure 6.7.

Appendix

In this appendix we prove the simplification of the bound on the relative effectiveness that was stated in equation (6.2.12).

Proposition 6.A.1

For integers $1 \leq r \leq k$,

$$B = \frac{k! (r-1)^{r-1} (k-r)^{k-r}}{(r-1)! (k-r)! (k-1)^{k-1}} \leq \begin{cases} k & \text{if } r=1 \text{ or } k \\ k^{3/2} (2\pi(r-1)(k-r))^{-1/2} & \text{otherwise.} \end{cases} \quad (6.A.1)$$

Proof

The left hand expression of (6.A.1) evaluates to k if $r=1$ or k . For the remaining cases $1 < r < k$, we use Stirling's approximation (Abramowitz and Stegun, 1970, eqn. 6.1.38)

$$x! = \sqrt{2\pi} x^{x+1/2} \exp\left(-x + \frac{\theta}{12x}\right) \quad (6.A.2)$$

valid for $x > 0$ and some $0 < \theta < 1$. Thus after simplification

$$B = \frac{k^{3/2} \left(\frac{k}{k-1}\right)^{k-1} \exp\left(\frac{\theta_1}{12k} - \frac{\theta_2}{12(r-1)} - \frac{\theta_3}{12(k-r)} - 1\right)}{\sqrt{2\pi(r-1)(k-1)}} \quad (6.A.3)$$

where $0 < \theta_1, \theta_2, \theta_3 < 1$, and we have

$$B < \frac{k^{3/2} \left(\frac{k}{k-1}\right)^{k-1} \exp\left(\frac{1}{12k} - 1\right)}{\sqrt{2\pi(r-1)(k-1)}} \quad (6.A.4)$$

Now consider

$$\begin{aligned} f &= \ln\left\{\left(\frac{k}{k-1}\right)^{k-1} \exp\left(\frac{1}{12k} - 1\right)\right\} \\ &= (k-1) \ln\left(1 + \frac{1}{k-1}\right) + 1/12k - 1. \end{aligned} \quad (6.A.5)$$

Taking the first three terms of the Taylor expansion of $\ln(1+x)$ about zero (valid here) since $1 < r < k$ implies $k > 2$ and

$1/(k-1) < 1$, we have

$$\begin{aligned} f &< (k-1) \left\{ \frac{1}{k-1} - \frac{1}{2(k-1)^2} + \frac{1}{3(k-1)^3} \right\} - 1 + 1/12k \\ &= \frac{-5k^2 + 8k + 1}{12k(k-1)^2} \end{aligned} \quad (6.A.6)$$

Thus for $k > 2$ we will have $f < 0$, whence

$$\exp(f) = \left(\frac{k}{k-1} \right)^{k-1} \exp\left(\frac{1}{12k} - 1 \right) < 1 \quad (6.A.7)$$

and our proposition follows from substitution of (6.A.7)

into (6.A.4).

Figure 6.1. The Moolgavkar-Venzon-Knudson (M-V-K) Two-Stage Birth-Death-Mutation Model. (λ_1 and λ_2 denote the transition rates from normal to intermediate cells and from intermediate to malignant cells respectively; α and β denote the birth and death rates of initiated cells.)

Figure 6.2. Relative Effectiveness of Exposures at Different Times in Multistage Models with k Stages Having the r^{th} Stage Dose-Dependent.

Figure 6.3. Equivalent and Time-Weighted Average Constant Doses for a Hypothetical Time-Dependent Dosing Pattern in a 6-Stage Model with the First Stage Dose-Dependent. ($R(\cdot)$, not to scale.)

Figure 6.4. Relative Effectiveness of Exposures at Different Times in the M-V-K Model with First Stage Dose-Dependent (X and δ constant).

Figure 6.5. Relative Effectiveness of Low Exposures at Different Times in the M-V-K Model with Dose-Dependent Promotion (X constant).

Figure 6.6. Human Liver Weight as a Function of Age.

Figure 6.7. Relative Effectiveness of Exposures at Different Times in the M-V-K Model with X Proportional to Human Liver Weight (δ constant).

Figure 6.8. Relative Effectiveness of Exposures at Different Times in the M-V-K Model with X Proportional to Human Liver Weight and δ Proportional to Human Liver Growth Rate.

Chapter 7.

FURTHER RESEARCH

Several difficult questions remain outstanding in the area of the reparametrization of linear compartmental models as discussed in Chapter 3. The parametrization that is presented there was discovered more or less by trial and error, so it is possible that improvements on it exist. For example, it would be extremely desirable to find a single parametrization that had uniformly low curvature, or to demonstrate that any such parametrization must lose some other desirable property such as partial linearity or the hierarchical ordering.

Another improvement on the results presented in Chapter 3 would be a single parametrization which handled all cases of more than two eigenvalues, including eigenvalues of triple or higher multiplicity. This would seem to be of somewhat limited practical value, as observable data would rarely be sufficient to warrant such a model, but it would be interesting nevertheless.

The exact algorithmic solutions for the steady-state of Michaelis-Menten networks that were presented in section 4.2 can probably be extended to somewhat more complex networks. For example, purely linear networks can all be solved, even though they are not of the form of the ordered compartmental systems discussed there. One would expect that some networks consisting of mixtures of linear and non-linear transitions could thus be solved.

The proof of the convergence of the steady-state approximations of section 4.3 was somewhat unsatisfying in that it did not specify a method to calculate the range of doses for which it would converge, nor did it produce an estimate of the residual error at each step of the algorithm. Both of these values would be useful in a practical implementation of the method.

Similar problems remain unresolved in the area of low-dose approximations considered in section 4.4. In particular, one wonders about the accuracy of the low-dose approximations. Extending these approximations into higher dose ranges might also be possible. It seems likely that as paths saturate there will be qualitative changes in the behaviour of a Michaelis-Menten system; at what doses do

these changes occur?

Of interest in the area of dose-response curves for mixtures (Chapter 5) is the problem of modelling or estimating the mixture distributions that actually exist. For example, models such as the Birth-Death-Mutation model may be close enough to reality to allow information that is experimentally obtainable to be used to estimate the values and variabilities of component parameters. It may be possible to integrate these distributions in the form of a mixture distribution as considered in that chapter.

The estimation problem also seems to be the major outstanding problem in the risk assessment of time-dependent dosing patterns. It should be possible to estimate relative effectiveness functions directly from suitable data. This would be of considerable value by itself, in that it would allow the determination of particularly susceptible age groups for particular carcinogens if such exist. It might also have the indirect benefit of confirmation or contradiction of particular models of carcinogenicity.

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