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□ Dose-response data for many chemical carcinogens exhibit multiple apparent concentration thresholds. A relatively small increase in exposure concentration near such a threshold disproportionately increases incidence of a specific tumor type. Yet, many common mathematical models of carcinogenesis do not predict such threshold-like behavior when model parameters (e.g., describing cell transition rates) increase smoothly with dose, as often seems biologically plausible. For example, commonly used forms of both the traditional Armitage-Doll and multistage (MS) models of carcinogenesis and the Moolgavkar-Venzon-Knudson (MVK) two-stage stochastic model of carcinogenesis typically yield smooth dose-response curves without sudden jumps or thresholds when exposure is assumed to increase cell transition rates in proportion to exposure concentration.

This paper introduces a general mathematical modeling framework that includes the MVK and MS model families as special cases, but that shows how abrupt transitions in cancer hazard rates, considered as functions of exposure concentrations and durations, can emerge naturally in large cell populations even when the rates of cell-level events increase smoothly (e.g., proportionally) with concentration. In this framework, stochastic transitions of stem cells among successive events represent exposure-related damage. Cell proliferation, cell killing and apoptosis can occur at different stages. Key components include:

1. An effective number of stem cells undergoing active cycling and hence vulnerable to stochastic transitions representing somatically heritable transformations. (These need not occur in any special linear order, as in the MS model.)
2. A random time until the first malignant stem cell is formed. This is the first order-statistic, T = min{T1, T2, . . . , Tn} of n random variables, interpreted as the random times at which each of n initial stem cells or their progeny first become malignant.
3. A random time for a normal stem cell to complete a full set of transformations converting it to a malignant one. This is interpreted very generally as the first passage time through a network of stochastic transitions, possibly with very many possible paths and unknown topology.

In this very general family of models, threshold-like (J-shaped or multi-threshold) dose-response nonlinearities naturally emerge even without cytotoxicity, as consequences of stochastic phase transition laws for traversals of random transition networks. With cytotoxicity present, U-shaped as well as J-shaped dose-response curves can emerge. These results are universal, i.e., independent of specific biological details represented by the stochastic transition networks.

Keywords: Low dose nonlinearity, U-shaped dose-response curve, stochastic models of carcinogenesis, sharp transition threshold

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1. INTRODUCTION AND MOTIVATION

What is the least amount of knowledge about carcinogenic processes needed to predict their corresponding dose-response relations? Historically, some of the most widely applied dose-response models have been based on very simple, generic postulates about carcinogenesis. For example, the linearized multistage model that dominated regulatory risk assessment of carcinogens in the US for many years is based on the Armitage-Doll conceptual model of cancer (e.g., Brown and Chu, 1987), in which originally "normal" stem cells undergo somatically heritable transformations, eventually producing a fully malignant cell that can develop into a clinically observed cancer after some delay, unless death from other causes occurs first. The more recent Moolgavkar-Venzon-Knudson (MVK) two-stage stochastic model of carcinogenesis emphasizes two rate-limiting transformations, from "Normal" to "Initiated" (also called "pre-malignant") and from "Initiated" to "Malignant", while incorporating proliferation and cell death of initiated cells at rates that can depend on exposures. In both models and several variations (e.g., Little *et al.*, 2002), exposure can also affect the transformation rates taking cells at each stage to the next.

Such models have been popular in part because they lead to parametric families of dose-dependent, age-specific hazard functions for cancer that can be fit to many epidemiological and rodent cancer bioassay data sets without having to understand all the details of how transformations take place, or even necessarily what specific cell populations correspond to each model stage. Exposure and tumor data suffice for fitting the models. On the other hand, the same models provide clear biological interpretations and explanations for much experimental evidence, including initiation-promotion-progression experiments in which applying carcinogens over time in different orders creates very different carcinogenic responses, essentially because creating initiated cells before amplifying them with a promoter that stimulates their proliferation, or completing their transformation to malignancy with a progressor, is far more effective in producing tumors than reversing this order.

The conceptual value and practical successes of these stochastic transition models can often be increased by making modifications to capture important phenomena observed in epidemiology and experimental carcinogenesis. Among these are:

- Importance of proliferation of normal cells in increasing cancer risks (Holt, 1997 for radiation). Many chemical carcinogens have been found to increase tumor rates in experimental animals only in situations that also cause cytotoxicity and regenerative hyperplasia or compensating proliferation of apparently normal cell populations in response to the toxic injury. Examples include chloroform, diesel

exhaust, formaldehyde, and many others. When such compensating proliferation is a prerequisite for chemically induced carcinogenesis, traditional linearized multistage modeling may over-estimate risks at low concentrations by more than five orders of magnitude (Larson *et al.*, 1996), or predict significant risks at low concentrations even if none truly exists (Constan *et al.*, 2002). Thus, dose-response models that account for the role of normal stem cell proliferation and kinetics following cytotoxic damage may be needed to obtain realistic risk estimates for some chemicals.

- *Carcinogenic thresholds* in dose rate (e.g., concentration for inhaled lung carcinogens) and/or duration of exposure

Table 1 illustrates the idea of carcinogenic thresholds with experimental data for isoprene. Liver and lung adenomas and carcinomas are only significantly elevated at concentrations above 70 ppm. In this data set, 140 ppm is the smallest concentration for which significant increases were observed. Similarly, histiosarcomas exhibit an apparent response threshold between 140 and 280 ppm. These thresholds are specific to concentration, rather than to cumulative exposure. For example, doubling concentration (from 70 ppm to 140 ppm between exposure groups 3 and 5) increases liver adenomas from 0.29 to 0.44, whereas, doubling weeks of exposure (from 40 to 80 between exposure groups 3 and 4) does not increase risk significantly at any site, and even appears to reduce it. Similarly, quadrupling exposure concentration from 70 ppm to 280 ppm while quartering exposure duration from 80 weeks to 20 weeks unambiguously increases tumor risk (compare exposure groups 4 and 6), even though cumulative exposures are identical.

TABLE 1 Results of a stop-exposure experiment for Isoprine in male B6C3F1 mice

Group	ppm	weeks	hr/day	Liver adenomas	Lung adenomas	Other adenomas	Liver carcinomas	Lung carcinomas	Histiosarcomas
1	*0*	*0*	*8*	0.22	0.22	0.14	0.18	0	0
2	*10*	*80*	*8*	0.24	0.32	0.12	0.12	0.02	0.04
3	*70*	*40*	*8*	0.29	0.16	**0.30***	0.22	0	0.04
4	*70*	*80*	*8*	0.30	0.08	0.18	0.18	0.04	0.04
5	*140*	*40*	*8*	**0.44***	0.20	**0.28***	0.20	0.02	0.02
6	*280*	*20*	*8*	0.36	0.32	**0.36***	0.24	0.06	**0.16***
7	*2200*	*80*	*4*	**0.42***	0.30	**0.56***	0.30	0.06	**0.14***
8	*2200*	*40*	*8*	**0.57***	**0.59***	**0.65***	**0.37***	0.06	**0.14***

Explanation: Columns 2–4 summarize the exposures defining each dose group. The remaining columns show the fraction of animals in each dose group that were found to have each tumor type at necropsy. *Source:* Cox LA Jr, Bird MG, Griffis L. Isoprene cancer risk and the time pattern of dose administration. *Toxicology.* 1996 Oct 28;113(1–3):263–72.

* Tumor incidence rates in bold and marked with an asterisk are significantly greater than in the control group ($p < 0.05$ by Fisher's Exact Test).

Apparent carcinogenic thresholds or threshold-like nonlinearities can also exist for components of exposure duration. For example, at higher concentrations, doubling hours-per-day of exposure while halving weeks of exposure increases the risk of adenomas and liver carcinomas (compare exposure groups 7 and 8), even though cumulative exposures remain identical.

Such threshold-like behavior for exposure concentrations and durations occurs for many chemical carcinogens. It requires explanations that go beyond the usual multistage and MVK stochastic transition models with linear-in-dose transition rates, since both of those models predict that risk changes smoothly with the concentration and duration of internal doses received by target tissues or cell populations. Physiologically-based pharmacokinetic (PBPK) modeling shows that these internal dose attributes, in turn, are typically smooth (and, at low doses, approximately linear) functions of the concentration and duration of administered dose over the range of experimental data, even though induction, depletion, or saturation of enzyme-mediated processes can introduce important non-linearities at very high administered doses.

To better understand how threshold-like nonlinearities can occur at experimental or low doses even without nonlinearities in the PBPK component, this paper reexamines the mathematical foundations of stochastic transition models of carcinogenesis and discusses how natural (smooth, possibly linear no-threshold) dose-response relations at the level of individual cells can lead to the sharp transitions and threshold-type behaviors observed in some empirical dose-response relations for aggregate cell populations.

2. DETERMINISTIC THRESHOLDS IN STOCHASTIC TRANSITION SYSTEMS

The Symmetric Multistage Model

To build intuition, let us first consider a *symmetric multistage model* as follows: A cell line gradually accumulates transformations (e.g., somatically heritable mutations) from a set of K possible transformations. Transformations occur randomly and independently over time, i.e., each of the K transformations arrives according to an independent Poisson process, with (at least approximately) equal intensities, given by λ average occurrences per unit time for each of the K distinct transformations. (Transformations with intensities that are much greater than this common minimum value are not rate-limiting, and may be disregarded. Once any of the K transformations has occurred, we assume that it is permanent and irreversible. If a specific transformation occurs more than once, the occurrences after the first one are wasted, i.e., the cell genotype has already acquired that transformation and does not reach malignancy any quicker if it occurs again.) The cell line survives

for a finite lifetime of duration T. If all K distinct transformation occur before time T, then the cell line becomes malignant. Under these conditions, what is the probability that the cell line will become malignant before death at time T? And, if it does become malignant before time T, then what is the probability distribution for the time at which the first malignant cell is formed?

The somewhat surprising answer is that, for sufficiently large K, there is a "sharp transition" time such that the first malignant cell is very unlikely to be formed much sooner or much later than that time. In other words, a nearly deterministic occurrence time for the first malignant cell emerges simply as a consequence of there being many stages in this simple stochastic transition model.

THEOREM 1: In the completely symmetric multistage model, there is a "sharp transition" time* [given to a close approximation by $T^* \approx (1/\lambda)((\ln(K) + \gamma)$, where λ is the expected number of transformations events per unit time, i.e., their average occurrence rate; and γ = Euler's constant = 0.57721 . . .] such that: (a) The expected time until the first malignant cell is formed is T*; and (b) the coefficient of variation of the actual (random) time of formation of the first malignant cell, i.e., the ratio of its standard deviation to T*, approaches 0 for large K.

Proof: The expected *number* of transformation occurrences, including wasted (i.e., repeated) ones, until a malignant cell is formed (i.e., until all K transformations have occurred at least once) is given by the harmonic sum: $E(n^*) = K(1 + 1/2 + 1/3 + \ldots + 1/K) \approx K((\ln(K) + \gamma)$, where n* denotes the random number of the transformation occurrence event at which all K transformations are first completed and γ is Euler's constant, γ = 0.57721. . . . This follows from previously known results for the "Coupon Collector's Problem" with equal probabilities (e.g., Ross, 1996 p. 414; or Motwani and Raghavan, 1995) or for the maximum of K independent exponential random variables (e.g., Nelson, 1995, page 173). (Intuitively, it is motivated by the fact that any of the K transformations can occur first and be non-redundant, after which the probability that the next transformation is non-redundant drops to $(K - 1)/K$, then to $(K - 2)/K$, . . . , and finally, for the last transformation, to $1/K$.) The expected *time* until a malignant cell is formed is therefore $T^* = E(t^*) = E(n^*)/(K\lambda) \approx (1/\lambda)((\ln(K) + \gamma)$ where t* denotes the random time at which all K transformations are first completed and $K\lambda$ is the rate at which transformations events arrive (since each of K types independently arrives at rate λ.) This proves part (a) of the theorem. That the probability distribution of n* has a sharp concentration around E(n*) is proved in Motwani and Raghavan, 1995. Given this key result, hold n* fixed. The time until n* transformations

(including redundant ones) have occurred has a gamma distribution with mean $n^*/(K\lambda)$ and variance $n^*/(K^2\lambda^2)$, by a standard results for waiting times in Poisson arrival times and for the mean and variance of the gamma distribution (e.g., Ross, 1996, p. 18). The ratio of the standard deviation to the mean of this waiting time is therefore $(n^*)^{-1/2}$ ($[K((\ln(K) + \gamma)]^{-1/2}$, yielding part (b).

An interesting, and perhaps unexpected, aspect of this result is that it establishes a form of nearly deterministic behavior for a stochastic system: if the sharp transition time T* is smaller than the death time T, then formation of a malignant cell by time T is almost certain; otherwise, it is very unlikely. (This qualitative behavior is typical of what is sometimes called a 0–1 law in stochastic processes.) If K is not large enough to guarantee a sharp transition at time T*, then the qualitative behavior can be generalized as follows: for any $\varepsilon > 0$, no matter how small, there is an interval of times $[T^-, T^+]$ such that the probability of a malignant cell being formed before T^- or after T^+ is less than ε, i.e., the cumulative probability distribution for the occurrence time of the first malignant cell increases from almost 0 to almost 1 over this interval. As K increases, the width of this interval shrinks toward zero, with T^- and T^+ approaching a common value, T*.

In this simple model, exposure to carcinogens can increase cancer risk either by increasing λ or by decreasing K (in effect, by completing some transformations relatively quickly, so that they are removed from the rate-limiting set). Either reduces T*, making formation of malignant cells prior to death of the cell line more likely. If the effect of a carcinogenic exposure is to reduce K, the number of remaining transformations required to reach malignancy, then the lifetime probability of tumor, $\Pr(T^* < T)$, will increase in discrete steps (corresponding to transformations completed and no longer on the critical path) that may be insensitive to the detailed exposure pattern used to reduce K.

Generalizations

Of course, the symmetric multistage model is not realistic as a general model of carcinogenesis. Initiation-promotion experiments have established that there is asymmetry in the set of changes leading from normal to malignant cells, with initiation changes preceding progression stages. However, the symmetric case illustrates some ideas that hold more generally.

Let $\mathbf{x}(t) = [x_1(t), x_2(t), \ldots, x_K(t)]$ denote the "state" of a cell at time t, with $x_j(t) = 1$ if some specific fact j is true of the cell at time t and $x_j(t) = 0$ otherwise. Thus, the Boolean vector $\mathbf{x}(t)$ indicates which facts are true of the cell at time t. Let $F(\mathbf{x})$ be a monotonically increasing 0-1 function of $\mathbf{x}$, with $F(\mathbf{0}) = F(0, 0, \ldots, 0) = 0$ and $F(\mathbf{1}) = F(1, 1, \ldots, 1) = 1$, indicating whether some global property holds for the cell. This might be a

property such as that it has previously reached the "initiated" stage, that it has died, or that it has become malignant. (Although a cell can make a transition from initiated to malignant, we model this as setting the bits for "reached initiated" and "reached malignant" from (1, 0) to (1, 1) respectively, to preserve the convention that the cell only makes irreversible transitions among monotonically increasing states.) The facts are described ("oriented") in such a way that having more of them true never decreases the value of $F(x)$, while $F(x) = 0$ when none of the facts in $\mathbf{x}$ is true and $F(\mathbf{x}) = 1$ when all of the facts in x are true. For example, $\mathbf{x}$ might indicate which transformations leading from a normal stem cell to a malignant cell have taken place. In this family of models, the global property of interest is assumed to be determined by the truth values of the finite set of facts summarized in $\mathbf{x}$. Finally, suppose that $\mathbf{x}(t)$ increases monotonically (although, perhaps, randomly) over time, i.e., $\mathbf{x}(t + s) \geq \mathbf{x}(t)$ for any $s \geq 0$ (where the inequality holds component by component), so that facts that once become true remain true thereafter. This captures the idea of irreversible transformations, e.g., somatically heritable mutations.

THEOREM 2: (a) If $F(\mathbf{x})$ is a monotone function of a Boolean K-vector $\mathbf{x}$ with $F(\mathbf{0}) = 0$ and $F(\mathbf{1}) = 1$, and if $\mathbf{x}(t)$ increases monotonically with time from $\mathbf{x}(0) = (0, 0, \ldots, 0) = \mathbf{0}$ to $\mathbf{x}(t) = (1, 1, \ldots, 1) = \mathbf{1}$ for all sufficiently large t then for any $\varepsilon > 0$, no matter how small, there is a finite *transition interval* of times $[T^-, T^+]$ such that $\Pr(F(\mathbf{x}(t) = 1) \leq \varepsilon$ for all $t \leq T^-$ and $\Pr(F(\mathbf{x}(t) = 1) \geq 1 - \varepsilon$ for all $t \geq T^+$. In other words, the probability that $F(\mathbf{x}(t)) = 1$ (i.e., that the global property of interest holds) increases from almost 0 before T^- to almost 1 by T^+. (Moreover, the ratio T^+/T^- is bounded by a finite constant that depends only on ε.) (b) If $F(\mathbf{x})$ is also a symmetric function of its arguments and all facts have the same probability densities for the random times at which they become true, then the width of the transition interval is $T^+ - T^- = c/\ln(K)$, where c is a constant that depends only on ε. This width approaches zero for large K, i.e., the transition time has a sharp threshold.

Proof: Part (a) follows from a theorem of Bollobas and Thomason, 1986, also discussed by Friedgut and Kalai, 1996, p. 2994, upon replacing identical probabilities for each $\Pr(x_j = 1)$ with monotonically increasing functions of time between $\Pr(x_j = 1) = 0$ at time 0 and $\Pr(x_j = 1) = 1$ at some later time. This guarantees that K times t_j can be found such that all $\Pr[x_j(t_j) = 1]$ are equal, allowing the theorem of Bollobas and Thomason, 1986 to be applied. Part (b) is a reinterpretation of a sharp threshold result proved as Theorem 2.1 by Friedgut and Kalai, 1996.

Example: Mitotic Spindle Poison Thresholds

As a practical application, consider the probability that a specific cell undergoes mitotic spindle disruption leading to aneuploidy during mito-

sis in the presence of an aneugen. If at least some large number, K, of target sites (e.g., sulfhydryl groups on tubulin) must be bound by reactive metabolite molecules (e.g., *p*-benzoquinone, for benzene) during mitosis to disrupt spindle formation, and if metabolite molecules arrive at an average rate per site per minute that is proportional to their concentration in the target tissue, then part (b) of Theorem 2 implies that disruption will occur with high probability in the required time window if and only if the concentration is sufficiently great. (Interpret $\mathbf{x}(t)$ as the vector indicating which sites have been bound by time t.) In effect, there will be a critical concentration below which disruption does not occur and above which it occurs almost certainly.

Example: Receptor-Mediated Response Thresholds

Suppose that a cell has a large number of cell surface receptors, of which at least K must be bound simultaneously by ligand molecules (perhaps a chemical from an environmental source that mimics a natural cell signaling molecule) in order to trigger a particular response. Let c denote the average concentration of ligand molecules in the cell's environment, starting at time t = 0 and ending at time T, with concentration equal to zero outside this exposure interval. For simplicity, express c in units of expected arrivals of molecules at receptor sites per unit time. Let $p_j(t) = 1 - \exp^{-ct}$ be the corresponding probability that receptor j has become bound by time t. (Thus, $p_j(t)$ is the expected value of $x_j(t)$, where $x_j(t) = 1$ if receptor j has become bound by time t, else $x_j(t)$,= 0. We assume that binding times are relatively long, and so may be interpreted as being "irreversible" for purposes of this analysis.) Then $\mathbf{x}(t)$ summarizes which receptors have become bound by time t; $F(\mathbf{x}(t)) = 1$ if this number exceeds K and is 0 otherwise, indicating whether a cell-level response has been triggered; and part (b) of Theorem 2 implies that for large K and fixed T, the probability of triggering a cell response will be close to 0 for all concentrations below some critical concentration, c*, and will be close to 1 for concentrations greater than c*.

Actually, in this case, it is the area-under-curve (AUC) product (c x T) that matters: for large K, the response probability undergoes a sharp transition from 0 to 1 as AUC = cT increases past some threshold level AUC*. For a fixed exposure duration, such as T = 4 hours per day, the critical concentration is: c* = AUC*/T. If exposure occurs once per day for some number of hours of duration, with full recovery between consecutive days, then this simple model predicts that doubling the hours-per-day of exposure for a fixed concentration might have a relatively large effect (if $2cT > AUC^*$ but $cT < AUC^*$), while doubling the total number of days of exposure to the original concentration might have no effect (if $cT < AUC^*$ on each day). This is similar to the lung adenoma response between groups 7 and 8 in Table 1, which roughly doubles when hours-per-day of exposure is doubled and number of days of exposure is halved.

Of course, any such critical AUC-per-day threshold, AUC*, would presumably provide at best only part of the explanation for lung adenomas, as other mechanisms, such as compensating proliferation in response to cytotoxic damage, can also be important. Even if such a threshold for AUC-per-day does exist, it need not have anything to do with receptor-mediated responses, as many other phenomena also might satisfy the conditions of Theorem 2 and produce sharp transition intervals.

Example: Compensating Proliferation Thresholds

Suppose that the stem cells in an unstressed tissue are on average equally likely to self-renew (adding a net of one new stem cell) or to differentiate into two non-stem daughter cells (subtracting a net of one stem cell). When the stem cell population becomes depleted by cytotoxicity or other means, the stem cell proliferation rate increases to a maximum, e.g., with a 0.6 probability of renewal *vs.* only 0.4 of differentiation. This maximum stimulation to proliferate occurs if and only if the stem cell population is depleted by at least a certain fraction of its normal value, say, K/N, where N is the normal size and K is the number that must be simultaneously absent to achieve maximum stimulation. If a brief cytotoxic dose kills or removes each stem cell with probability p, then Theorem 2 (with $\mathbf{x}(t)$ summarizing which stem cells remain and which have been removed) implies that maximum compensating proliferation will occur if and only if p is greater than some critical value, p*. (Of course, partial compensation may occur at lower doses that remove fewer than K stem cells.)

In general, Theorem 2 applies to many settings in which occurrence of a global outcome or property requires that at least K independent events must occur within some time window of length T. If the probability of each event occurring by time T is p (which may depend on exposure concentration or more generally on exposure history over the interval from t = 0 to t = T), and if the number of events K is large, then the global outcome is almost certain to occur if p is greater than some critical value p*, and otherwise is almost certain not to occur. This threshold property does not depend on the nature of the events: it is a universal property of systems with properties that can be described by monotone symmetric functions of K independent Bernoulli (0-1) random variables.

Example: Transformations with Precedence Constraints: Event Trees and Transition Networks

A useful generalization of many multistage models is to allow for more than one possible sequence of events (e.g., somatically heritable transformations, epigenetic events) leading from normalcy to malignancy, while keeping the feature that some events must be completed before others can occur. A general process of this type can be described as an *event tree*, explicitly enumerating the possible sequences of events that

take a normal stem cell to a malignant one. Each node in the tree corresponds to the sequence of events that have occurred so far, and the successors of each node are the events that can occur next, corresponding to branches out of the current node. The branch probabilities at each node complete the specification of the process. (A directed acyclic graph (DAG) indicating allowed transitions among events, along with their probabilities, i.e., a *stochastic transition network* (STN), often provides a more concise representation of the same information. However, the tree provides a clear, useful conceptual model of multiple alternative paths and precedence partial ordering constraints among events.)

The time at which a malignant cell is first formed can be interpreted as a *first passage time* (Harrison and Knottenbelt, 2001) through the tree or through its corresponding stochastic transition DAG. Let $\mathbf{x}(t)$ indicate which nodes or which transition arcs have been traversed by time t, and let $F(\mathbf{x})$ indicate whether a tip of the tree (or a "malignant" node) has been reached when the state is $\mathbf{x}$. Then $\mathbf{x}(t)$ is monotonically increasing in time, part (a) of Theorem 2 holds, and there is a finite transition interval that contains the first passage time, i.e., the probability that the first malignant cell has been formed essentially goes from 0 to 1 over this interval. The transition need not be sharp, however, unless the event tree has internal symmetries. The total symmetry in part (b) of Theorem 2 is stronger than necessary. For example, if there is a sharp threshold for the time to bring a cell from node A to node B in a DAG (via any of several paths), and another sharp threshold for the time to bring it from B to C, then there is a sharp threshold for the passage time from A to C, despite the asymmetries that A precedes B and B precedes C.

More generally, Theorem 2 can be strengthened and extended in several ways to apply to more general classes of systems, e.g., to characterize the distribution of first-passage times through transition graphs that are not necessarily acyclic, but that eventually lead to malignancy, starting from any other node, with probability 1. Such models can allow for some reversible transitions, e.g., reflecting fallible repair processes. However, Theorem 2 suffices for several interesting applications.

3. APPLICATIONS TO CARCINOGENESIS

In the MVK two-stage model of carcinogenesis, each of N normal stem cells may undergo initiation. Initiated stem cells may die, replicate, or undergo an additional transformation to become malignant. It is usually assumed that exposure results in biologically effective doses that increase the rates of initiation, initiated cell birth or death (e.g., promotion or apoptosis, respectively), and transformation of initiated to malignant cells (progression) in proportion to the dose. The transition interval framework in Theorem 2 suggests some alternatives to this proportional-increase assumption. In particular, rather than essentially unpre-

dictable (exponentially distributed) transition times for each cell, transition times may be confined to certain highly probable intervals, with the widths of these intervals being small in some situations, e.g., for transitions described by monotone symmetric functions of many independent, identically distributed Bernoulli random variables.

Suppose that, for a given exposure history, exposure-induced initiation of susceptible stem cells can occur in the transition interval (t1, t2), i.e., between ages t1 and t2 years, while progression to malignancy is likely to take an additional amount of time of between t3 and t4 years, corresponding to a transition interval of length t4 – t3. [Both initiation and malignancy are here considered as irreversible events in the stochastic evolution of a cell (or somatic cell line) from normalcy to malignancy, and the conditions of part (a) of Theorem 2 are satisfied, so that there is a transition interval for each event.] If further observations are censored by death at time T, then the probability density for the time of a malignant cell being formed prior to death will be concentrated in the interval [min{t1 + t3, T}, min{t2 + t4, T}]. Thus, if t1 + t3 > T, malignant cells will not arise; if t2 + t4 < T, then malignant cells may arise prior to t2 + t4, but not at older ages, i.e., the *hazard rate decreases with older ages* past some point, reaching 0 at age t2 + t4. Attaining this age without a malignant cell implies that no susceptible cells entered the pipeline during (t1, t2), so that none will emerge as malignant cells after t2 + t4. In all other cases, malignant cells may arise throughout old age, beginning at age t1 + t3 and continuing until death.

In this framework, exposure to carcinogens can have any of the following effects:

1. Reduce t1 and t2, i.e., shift the *initiation interval* between them [t1, t2], leftward toward earlier ages, by completing one or more of the transformations required for a normal cell to become initiated. If the number of such transformations, K, is small, then only a few such discrete leftward shifts are possible, corresponding to different transformations that can be completed by the carcinogen. Each such shift stochastically decreases (Ross, 1996) the remaining time to malignancy.
2. Shift the *progression interval* [t3, t4] leftward. This occurs if the exposure completes one or more transformations in an initiated cell, stochastically decreasing the remaining time until first passage of that cell (or its progeny) to malignancy.
3. *Amplify the population of initiated cells.* If each initiated cell has probability P of becoming malignant before death at time T, perhaps by traversing some path through a complicated stochastic transition network, then a population of I initiated cells has probability $1 - (1 - P)^I$ of generating at least one malignant cell before T. Thus, increasing I increases the lifetime probability of cancer.

4. *Amplify the population of normal stem cells* that are susceptible to initiating transitions. Cytotoxic effects on normal cell populations, which typically have concentration and/or AUC thresholds, can lead to compensating proliferation of normal stem cells and increased flux (after a delay of t2 – t1) into the initiated compartment, again increasing the average value of I.
5. Combinations of the above.

If the exposure-driven transitions in (1) or (2) themselves have sharp transition thresholds for an exposure variable such as AUC, i.e., if they occur with high probability if and only if dose exceeds some critical level, AUC* ppm-hours per day, as in the receptor-mediated response example above, then the possible discrete leftward shifts in [t1, t2] will be triggered by exposures above this threshold, and otherwise will not occur. There may be several different such dose-response thresholds. This threshold behavior for increases in risk might generate data similar to the pattern for histiosarcomas and lung carcinomas seen in Table 1, having an apparent concentration threshold between 140 ppm and 280 ppm for 8-hour/day exposures.

Importantly for practical data analysis, the increased risk from exposure in this transition interval framework is only sensitive to details of exposure (e.g., the concentration) over the range of the transition interval(s): otherwise, details of exposure are irrelevant for predicting response. All that matters is which events get triggered with high probability by the exposure pattern.

U-Shaped Dose-Response Relations

Suppose that a normal stem cell (or somatic cell line) must successfully traverse a stochastic transition network (STN) to become malignant. Paths through the network correspond to sequences of events that transform a normal genotype to a malignant one. The network may consist of two or more stages, e.g., first with multiple possible paths leading from normal to initiated, and then with multiple other paths leading from initiated to malignant. While such a staged structure can potentially simplify analysis of the effects of exposures on the distribution of first passage times through the network, it is not necessary for the following analysis.

Consider the fates of normal stem cells with finite lifetimes, T (e.g., due to apoptosis, cytotoxic exposures, clonal succession, etc.) entering the STN. (T may be a random variable, with different realized values for different cells.) Starting from the initial node, which might be called NORMAL, each cell progresses through the network by making stochastic transitions, eventually reaching the final node (MALIGNANT) with some probability unless it dies or differentiates first, i.e., unless elapsed time T occurs before malignancy. In this setting, any condition that shortens T

(or stochastically reduces it, if T is a random variable), including exposure to a cytotoxic carcinogen, will tend to reduce the probability of successful traversal of the network, other things (i.e., transition rates) being held equal. In contrast to K-stage models in which exposure can only hasten the transition of cells toward malignancy, in this framework, exposure can reduce the probability that a cell survives to reach malignancy.

For example, if an increase in weeks of exposure decreases T, e.g., by increasing the probability of cell death per week, but without further increasing transition rates in the STN (e.g., due to the threshold effects described above, and because exposure intensity in ppm-hours/day remains unchanged), then the increase in weeks of exposure would *reduce* the probability of carcinogenesis per normal stem cell entering the STN. If this reduction outweighs any increase in the flux of normal stem cells entering the STN per unit time to compensate for reduced T, then the net result would be a reduced risk of malignant cells. For example, if the number of cells per unit time increases homeostatically just enough to offset the shorter life per cell, thus maintaining cell population sizes, then the lifetime risk of cancer could be reduced, with a larger number of shorter-lived cells having less probability of successfully percolating through the STN than a smaller number of longer-lived cells. Such a phenomenon might be consistent with the observed reduction in lung and other adenomas in Table 1 between groups 3 and 4 if the time scale for first passage times through the STN is on the order of days to weeks. Group 4 has twice the weeks of exposure of group 3, yet a significantly lower rate of adenomas. (The difference is not due to censoring by early mortality, as these doses are well below lethal levels.) To test the hypothetical explanation that this is because T is reduced, one might investigate the cell kinetics (average life spans and fluxes) of affected cell populations to determine whether the average life spans per cell are in fact significantly reduced by additional weeks of exposure.

In STNs where relatively many stem cells traverse the first few nodes and relatively few penetrate much deeper into the network, energetically costly defenses (e.g., detection and repair or apoptosis mechanisms for damaged cells) may tend to be distributed primarily among the most frequently traversed, early nodes. In effect, if blocking cancer confers some evolutionary advantage, then defenses that block a high proportion of potential cancers may have been selected, while lower-payoff late defenses may not. In this case, exposures that reduce T, tending to keep cells in the earlier, relatively well-defended parts of the STN, may be especially likely to reduce cancer risk. Expressed in terms of an MVK two-stage model, U-shaped dose-response relations can arise not only by exposures that kill initiated cells (Holt, 1997; Bogen, 2001), but also by exposures that inhibit the fluxes of normal to initiated cells and/or of initiated to malignant cells.

4. CONCLUSIONS

We have presented a generalization of stochastic transition models of carcinogenesis that replaces the individual transitions in such models (e.g., from normal to initiated cells or from initiated to malignant cells) with stochastic transition networks (STNs). Exposures can affect cells not only by linearly increasing transition rates and by amplifying initiated and/or normal stem cell populations (as in Holt, 1997), but also by causing some transitions to happen with certainty (in effect, making their rates infinite) and by shortening the time T that cells have to complete a passage through the STN before dying or differentiating. More generally, we have considered models in which facts about a cell or cell line (or other system) can become true at random times, and malignancy occurs as soon as some subset of facts has become true, analogous to a minimal path set in systems reliability theory. There may be many different subsets of facts that jointly suffice to entail malignancy, and the first one completed then determines the time at which a malignant cell is first formed.

Within this framework, we applied known results from random graph theory to deduce two useful mathematical properties: (1) The existence of a finite *transition interval* for occurrence of malignancy (or for the first passage time of a normal cell through an STN), with the probability of malignancy increasing from 0 to 1 (to any desired degree of precision) over that interval; and (2) The existence of *sharp thresholds* for transitions, i.e., transition intervals whose widths approach zero, for systems with global symmetry and large numbers of components. We observed that the symmetry condition is needlessly strong, i.e., sharp transition thresholds may hold even in multistage (asymmetric) models with STNs rather than single transitions between consecutive stages, provided that each STN has a sharp transition threshold. These results also hold for transitions expressed as functions of exposure concentration, or AUC (ppm-hours) per day of exposure, as well as for time.

The STN and transition interval framework developed in this paper predicts the possibility of some qualitative behaviors seen in stop-exposure experimental data for chemical carcinogens. These include:

- The possibility of age-specific hazard functions that decrease with age after some point.
- Sharp threshold or more gradual "J-shaped" portions of dose-response curves, where response probabilities increase suddenly as exposure concentration or hours-per-day of exposure increase relatively slightly near a critical threshold value (e.g., histiosarcomas between 140 ppm and 280 ppm in Table 1.)
- Flat, L-shaped, or U-shaped relations between total dose and risk, when increasing weeks of exposure has little effect or even suppresses cancer risk (as for groups 3 and 4 in Table 1. See also groups 7 and 8 for what

may be a combination of a J-shaped response to hours-per-day of exposure, and hence to ppm-hours/day, with a flat or U-shaped response to weeks of exposure.)

The possibility of these qualitative behaviors emerges from relatively simple, high-level assumptions about the stochastic transition networks and resulting transition intervals involved. For example, the sharp transition threshold results based on monotonicity, symmetry and a requirement that large numbers of binary facts must hold (exemplified by k-out-of-n receptor sites becoming bound within a certain time window) are universal, in that they do not depend on the details of the facts in any way.

The main practical message from the mathematical modeling in this paper is that the abrupt transitions and multi-threshold type behaviors observed in some experimental cancer dose-response data can be explained as a natural consequence (an emergent property) of the complexity of pathways leading to carcinogenicity, even when individual events at the cell level occur at rates that vary slowly and smoothly (e.g., linearly) with exposure. When transitions from one cell type to the next can occur in many different ways, so that each transition can be thought of as a passage through a network with many possible paths through it, this complexity can result in abrupt transitions of the probability of successful passage through the network as a function of exposure or dose. Such abrupt transitions are an emergent property of a wide class of stochastic transition network models. The idea that complexity of possible transitions toward carcinogenesis can explain abrupt dose-response transitions is especially relevant for cancers with complex, stochastic etiologies, as indicated by tumors with different profiles of accumulated damage and no small set of necessary and sufficient changes. These include lung cancers (Wistuba *et al.*, 2001) and other cancers of importance in public health.

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