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Publication Date:
2006-01

Permanent Link:
https://doi.org/10.3929/ethz-b-000158079 →

Originally published in:
Toxicological Sciences 89(1), http://doi.org/10.1093/toxsci/kfj024 →

TOXICOLOGICAL SCIENCES **89(1)**, 331–337 (2006)
doi:10.1093/toxsci/kfj024
Advance Access publication October 19, 2005

Dose-Incidence Modeling: Consequences of Linking Quantal Measures of Response to Depletion of Critical Tissue Targets

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Received August 1, 2005; accepted October 10, 2005

In developing mechanistic PK–PD models, incidence of toxic responses in a population has to be described in relation to measures of biologically effective dose (BED). We have developed a simple dose-incidence model that links incidence with BED for compounds that cause toxicity by depleting critical cellular target molecules. The BED in this model was the proportion of target molecule adducted by the dose of toxic compound. Our modeling approach first estimated the proportion depleted for each dose and then calculated the tolerance distribution for toxicity in relation to either administered dose or log of administered dose. We first examined cases where the mean of the tolerance distribution for toxicity occurred when a significant proportion of target had been adducted (i.e., more than half). When a normal distribution was assumed to exist for the relationship of incidence and BED, the tolerance distribution based on administered dose for these cases becomes asymmetrical and logarithmic transformations of the administered dose axis lead to a more symmetrical distribution. These linked PK–PD models for tissue reactivity, consistent with conclusions from other work for receptor binding models (Lutz *et al*., 2005), indicate that log normal distributions with administered dose may arise from normal distributions for BED and nonlinear kinetics between BED and administered dose. These conclusions are important for developing biologically based dose response (BBDR) models that link incidences of toxicity or other biological responses to measures of BED.

Key Words: **dose-incidence relationship; logarithm; individual susceptibility; biologically effective dose; pharmacodynamic modeling; pharmacokinetic modeling.**

Toxic responses in an exposed population arise from a series of steps that involve level of exposure, absorption of chemical into the body, delivery of chemical to sensitive tissues, interactions of these chemicals or their metabolites with biological targets in tissues, and progression of these cellular interactions, eventually leading to an increase in the proportion of individuals with an adverse response. Over the past 25 years, there have been steady advances in pharmacokinetic (PK) models that predict tissue concentrations of toxic chemicals and metabolites for many families of chemicals and simulate the interactions of these chemicals with tissue constituents (see Reddy *et al*., 2005). The subsequent steps, from these interactions to the expression of toxicity, have not received the same attention in relation to quantitative modeling.

Pharmacological and toxicological responses can be divided into two broad classes: reactivity and recognition. Reactivity refers to the alteration in chemical structures by covalent interactions with protein or DNA and to alterations in the cellular environment leading to processes such as protein denaturation. Recognition is the noncovalent interaction with specific receptors leading to occupancy of the receptor, which is followed by signal transduction events. The amounts bound covalently to tissues or noncovalently bound to specific receptors represent one possible measure of biologically effective dose (BED) of chemical at the target tissue. However, the expected relationship between toxicity and BED also depends on the mode of action for the toxic response. For carcinogenicity based on mutation, the amount of DNA adducts may be the appropriate metric for the BED, i.e., the risk of cancer may be related to the concentration of specific pro-mutagenic adducts in critical target genes (Lutz, 1979). For many other modes of action, toxicity is expected to ensue because of the alteration of important cellular functions by the loss of critical macromolecules or by the response to receptor-mediated processes. In these cases, the BED for specific responses is expected to be related to the extent of loss of the critical targets, i.e., to the proportion of cellular macromolecular target adducted or to receptor occupancy. The dose metrics for these BED take values between 0.0 (no critical site adducted or receptor bound) and 1.0 (all critical sites adducted or receptors bound).

Mechanistic pharmacodynamic (PD) models for toxic responses from chemical exposures have to link these continuous measures for the BED, i.e., the fraction depleted or fraction receptor occupancy, with the incidence of toxicity in a population. In these types of models, incidence is described by the distributions of susceptibility for response in the given

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population (Klaassen, 2001). In at least one example, developing a linked PK–PD model for chloroform toxicity, a quantal PD process, cell death, was linked to a measure of tissue dose, i.e., the rate of chloroform metabolism, using a normal distribution for repair capacity of cells (Reitz *et al*., 1990). In order to develop such linked PK–PD models for quantal responses at the cellular or organism level, it will be increasingly necessary to consider what relationship should be used to relate incidence with measures of BED. The traditional assumption of log normal[2] relationships of incidence to administered dose (Klaassen, 2001) needs to be revisited to include a broader consideration of both pharmacokinetic and pharmacodynamic factors that influence both tissue dose and BED.

In the accompanying FORUM article (Lutz *et al*., 2005), general principles of linking incidence to BED have been outlined and illustrated schematically for a receptor occupancy model. In this paper, we apply biochemical and PK principles to develop a linked dose-incidence model based on a normal distribution for toxicity in relation to a BED that is based on loss of some fraction of critical cellular targets. This line of research is a natural extension of consideration of target tissue dose or BED as the primary determinants of dose-incidence curves (Gehring *et al.*, 1976; Andersen *et al.*, 1987). The results of our modeling indicate that log normal distributions for incidence and administered dose are likely to arise from the combination of normal distributions for incidence and BED coupled with nonlinearities in relationship of BED and administered dose.

METHODS AND PROCEDURES

Model structures. PK–PD models for incidence in relation to administered dose include the changing dose level, the relationship of internal tissue dose to administered dose, the relationship of the BED to the internal dose metric, and the linkage of incidence to measures of BED. Administered dose, target tissue dose, and BED are all continuous functions in any individual in the population. In contrast, toxic responses in a population are described on a quantal scale, i.e., as proportions of populations showing a defined endpoint. Each individual, i.e., a cell in an organ, or an animal or human in a study population, either does or does not manifest the effect within a given period of observation. The dose-incidence relationship for biological effects in the population therefore reflects the tolerance distribution for toxicity in the investigated population. In our PK–PD models, these tolerance distributions for toxicity were assumed to be normally distributed with respect to BED, defined by a mean value (*mu*) and a standard deviation (*sigma*). In words, *mu* is that level of BED that results in half the individuals in the population showing the defined effect. Illustrating this relationship with the model for depletion of critical cell constituents, this means that 50% of the cells would be killed if the BED reaches the value *mu*.

Tissue reactivity. In the reactivity-based PK–PD incidence model, the measure of BED is the proportion of an essential cellular constituent (a target molecule, TM, such as glutathione or an important macromolecule) that is lost after treatment. This BED is a continuous variable ranging from 0.0 (no TM lost to reaction) to 1.0 (all TM lost by reaction during exposure). Here, we estimate the proportion of an essential cell constituent lost after a single dose of the toxic compound.

A cellular target molecule (TM) reacts with the toxic compound C with a second-order rate constant, k_{so}. The rate equation for loss of TM, where C and TM stand for the concentrations of the respective reaction components is:

$$dTM/dt = -k_{so} \times C \times TM \quad (1)$$

Integrating this equation for various doses gives,

$$\int d(TM)/TM = -k_{so} \times \int C\,dt \quad (2)$$

And,

$$\ln(TM_t/TM_0) = -k_{so} \times AUC \quad (3)$$

Substituting dose/clearance for AUC and rearranging for proportion of TM lost by reaction gives,

$$\ln(TM_0/TM) = (k_{so}/\text{clearance}) \times \text{dose} \quad (4)$$

$$(TM_0 - TM_{dose})/TM_0 = 1 - \exp\{-(k_{so}/\text{clearance}) \times \text{dose}\} \quad (5)$$

$$\text{BED (Proportion TM Lost)} = 1 - \exp\{-k' \times \text{dose}\} \quad (6)$$

The ratio (k_{so}/clearance) was set to 1.0 for our representative calculations. In addition, the dose can be back calculated based on the observed proportion of TM remaining by the simple formula:

$$\text{dose} = \log(TM_0/TM_{dose}) \quad (7)$$

With this model for reactivity, the BED (adducted fraction TM) is a nonlinear function of dose, asymptotically approaching 1.0 as dose increases, and follows an exponential relationship. For the reactivity based PK–PD model (Appendix A), curves were generated using proportion macromolecule depleted as the independent variable. For each value of the proportion depleted, dose calculated from Equation 7, and incidence was calculated using a normal tolerance distribution for the proportion of target molecule that was depleted. The resulting incidence curves could then be plotted with various x-axis variables, i.e., BED, administered dose, or logarithm of administered dose (see Figs. 1 and 3). Other PBPK models, such as one for steady-state extraction of vapors from the nose (Andersen *et al*., 1999), have also been implemented with dose, rather than time, as the independent variable.

Fitting log normal and normal distributions. Using approaches described in the accompanying FORUM paper (Lutz *et al*., 2005), distributions simulated from this PK–PD model that was linked to normal susceptibility distribution defined by mean *mu* and standard deviation *sigma* were fit to either truncated versions of cumulative log normal or a cumulative normal distribution, following $\Phi((\ln(\text{dose}) - m)/s)$ or $\Phi((\text{dose} - m)/s)$, where Φ is the cumulative standard normal distribution function. Fitting for *m* and *s* was limited to the central 95% incidence range ($0.025 < y < 0.975$) that was divided up into one thousand equidistant dose segments. The sum of the thousand differences between the incidence curve and the best fit were summed up. The result corresponds to an area, which was used as a measure of goodness of fit. Smaller areas represent better fits.

RESULTS

Converting Distributions Based on BED to Distributions Based on Administered Dose

The dose-incidence model for reactivity was run for various values of mean *mu* and standard deviation *sigma* for proportion target molecule adducted. The results are shown in Figure 1.

[2] Definition: If a variable is normally distributed when represented on a logarithmical scale, the distribution is called ''log normal''.

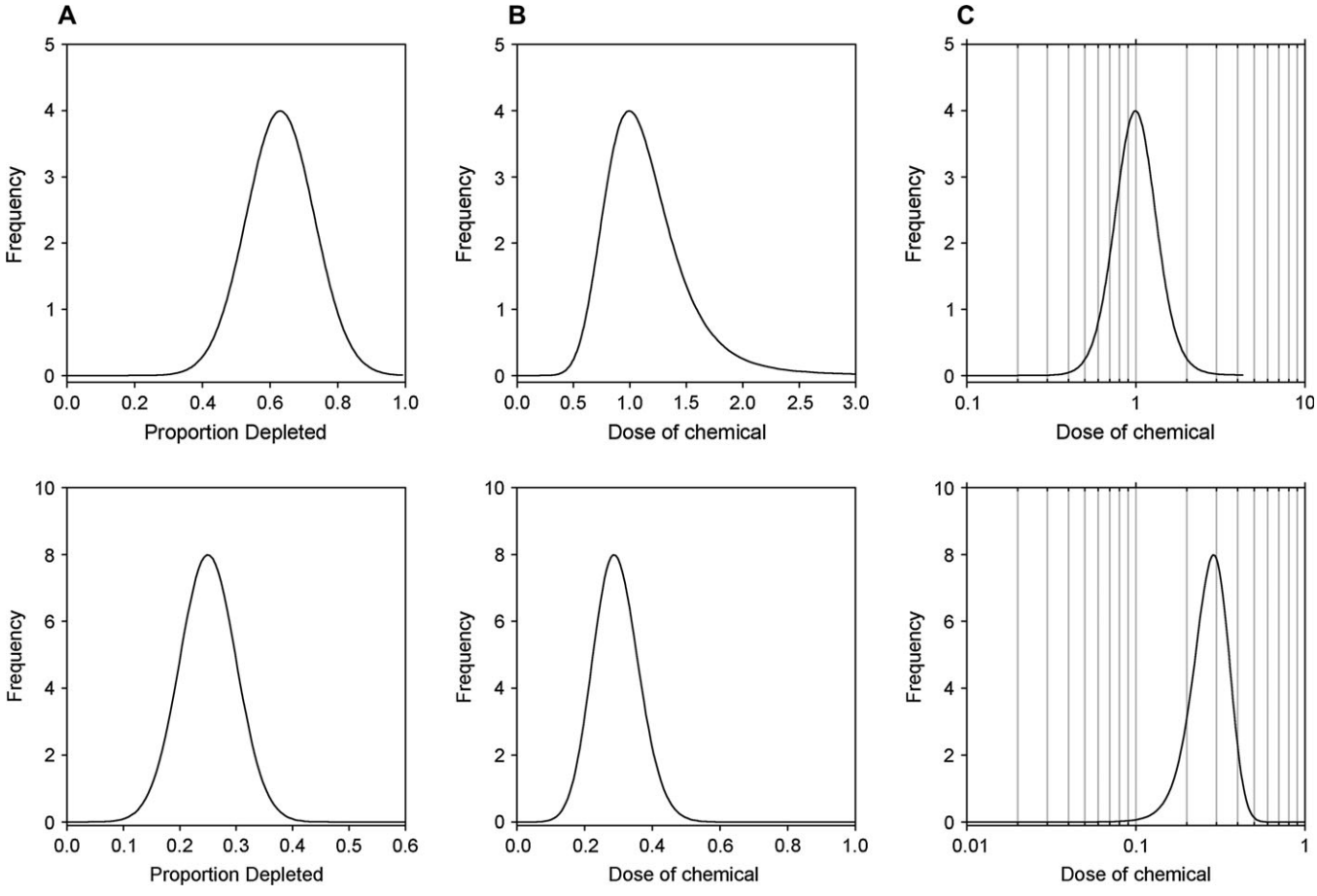

FIG. 1. Fractional dose-incidence relationships calculated for the PK–PD reactivity model. Frequency distributions are presented for two different independent variables. Panel A is the incidence curve based on the BED (i.e., the proportion depleted for reactivity); panels B and C show the distribution plotted versus administered dose, with arithmetic and logarithmic dose scaling, respectively. Top row of panels: $mu = 0.63$ and $sigma = 0.10$ for proportion depleted (the mean value that provides a distribution most closely resembling the log normal). Bottom row of panels: $mu = 0.25$ and $sigma = 0.05$.

For a high level of TM depletion required for toxicity (top row; $mu = 0.63$), the resulting tolerance distribution is displayed for incidence versus BED (entered as a normal distribution), versus dose, or versus log dose. Even though the assumed distribution with respect to BED was normal (Panel A), the incidence distribution based on administered dose was skewed (Panel B). When the calculated incidence was plotted against log dose, the distribution became more symmetrical (Panel C). For conditions where a much smaller proportion of depletion is associated with toxicity (mean $mu = 0.25$ and $sigma = 0.5$; bottom row of Fig. 1), the calculated normal distribution is more symmetric than the log normal distribution (Panel C). The differential behaviors for low and high values of *mu* arise due to the asymptotic nature of the relationship between BED and dose. In regions of low depletion, the relationship between proportion depletion (the BED) and dose is nearly linear. Here there is good mapping of the BED distribution on that for administered dose. In regions where the mean depletion for toxicity is large, the exponential relationship of BED to dose leads to increased skewing of the curve, as shown in Panel B of the top row.

Graphical representation of the fits to normal or log normal distributions as *mu* changes is shown in Figure 2. Fits to a log normal distribution were better than to a normal distribution for *mu*-values higher than 0.4, with an optimum at $mu = 0.63$ (center panel) Below $mu = 0.4$ the normal distribution was better, as seen in Figure 1, bottom, for $mu = 0.2$.

Numeric information on the fits is given in Table 1 for a wide range of *mu*-values (0.2 to 0.8) and for two different values of the standard deviation ($sigma = 0.05$ or 0.1). The values relate to relative areas between the dose-incidence curve (the full lines in Fig. 2) and the respective best-fitting curves (dashed for best-fitting log normal distribution, dotted for best-fitting normal distribution). The smaller the area, the better is the fit. Log normal distributions provided better fit for all $mu \geq 0.4$. The fit by a normal distribution deteriorated with increasing *mu*, while the fit by a log normal distribution showed an optimum, which was at a higher value for this function ($mu = 0.63$). For all calculations, k' was chosen equal 1. Using other values affected only the scaling of the dose axis but did not change the shape of the curves or the results of the comparative fitting.

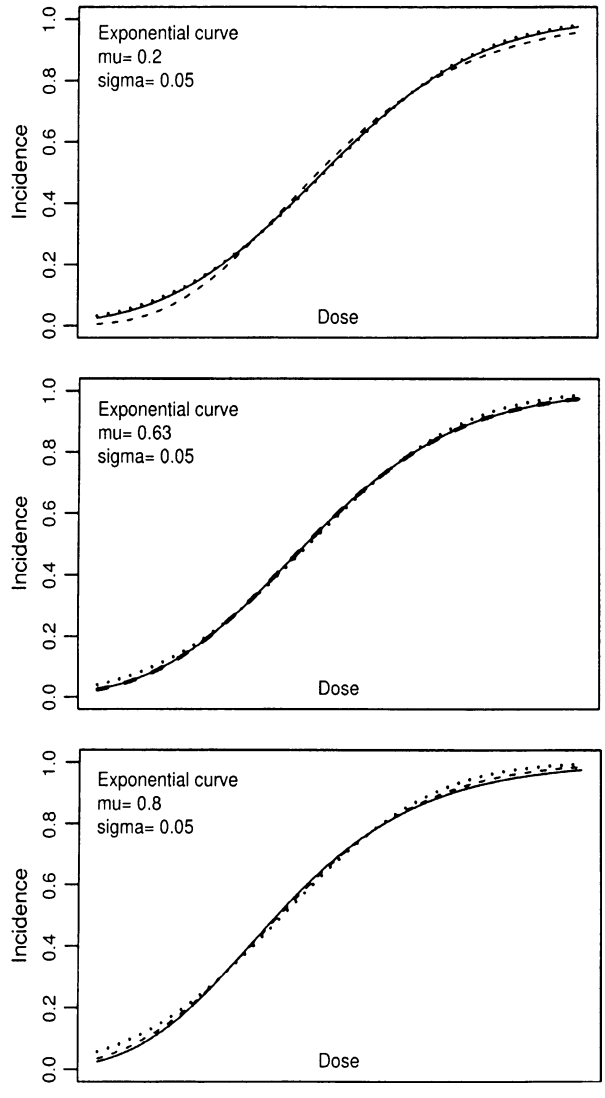

FIG. 2. Fitting a cumulative normal curve (dotted line) and a cumulative log normal curve (dashed line) to the dose-incidence curves generated by the PK–PD model for reactivity with BED as proportion of critical target molecules depleted (our exponential model). The three panels show fits for different means of the tolerance distribution (*mu* = 0.2, 0.63 [best fit], and 0.8, respectively). Standard deviation *sigma* = 0.05. "Incidence" is the proportion of individuals expected to be susceptible at a given "dose".

TABLE 1
Fit of Log Normal or Normal Distributions to the Results of a PK–PD Dose-Incidence Model for Depletion of Critical Cellular Target Molecule by Chemically Reactive Toxicants

	sigma = 0.05		*sigma* = 0.10	
	log normal	normal	log normal	normal
mu				
0.20	16.37	4.79	29.97	9.24
0.25	12.47	5.11	23.58	10.16
0.30	9.75	5.47	18.66	10.98
0.35	7.69	5.90	14.76	11.85
0.40	6.02	6.39	11.52	12.86
0.45	4.60	6.97	8.68	14.05
0.50	3.30	7.68	6.05	15.49
0.55	2.05	8.54	3.45	17.26
0.60	0.76	9.61	**1.84**	**19.49**
0.65	**0.72**	**11.00**	2.77	22.40
0.70	2.39	12.86	6.44	26.35
0.75	4.64	15.49	11.89	32.21
0.80	7.94	19.49	19.96	41.86

Note. Table values are the areas between the curves derived for the best-fitting cumulative log normal and the best-fitting normal dose-response curve from the exponential dose-incidence relationships for the reactivity PK–PD model linked to cumulative normal tolerance distributions for cell death (defined by mean *mu* and standard deviation *sigma*). The fitting was limited to the central 95% incidence range. Fits are better for the smaller values in the Table. The entry in bold highlight the values of mu where the calculated distribution in most similar to a log-normal distribution.

An increase in the standard deviation *sigma* from 0.05 to 0.1, i.e., an increase in the span of individual susceptibilities, resulted in a decreased fit for both the log normal and the normal curve. The worsening of fit was more pronounced for the normal fit. This observation indicates that, the wider the tolerance distribution, the more can be gained (in terms of fitting a cumulative normal curve to the data) by representing the dose-incidence data on a logarithmic dose scale.

Figure 3 shows similar behavior for the receptor-mediated model analyzed previously (Lutz *et al.*, 2005). Based on Michaelis–Menten kinetics for the dose–BED relationship, the log normal representation provided the best fit for *mu* = 0.5. The difference in the relationship of BED to administered dose for the two models is readily captured in plots of the hyperbolic (receptor occupancy) and exponential (reactivity) relationships shown in Figure 4. With unit values for the Michaelis constant in the occupancy model or for the constant multiplying dose in the reactivity model, the exponential curve of the reactivity model approaches the asymptote more quickly as dose increases. In either model, the best fit of the resulting distribution to a log normal occurred with unit values for the Michaelis constant or the constant in the exponential term in these normalized representations.

DISCUSSION

Log Normal Distributions

Log normal distributions have fairly consistently provided better descriptions of dose response curves for incidence of toxicity in populations of animals than have normal distributions. On the surface, the success of any consistent relationship of toxicity with administered dose is not at all expected based on modern concepts in toxicology. Administered dose is, at

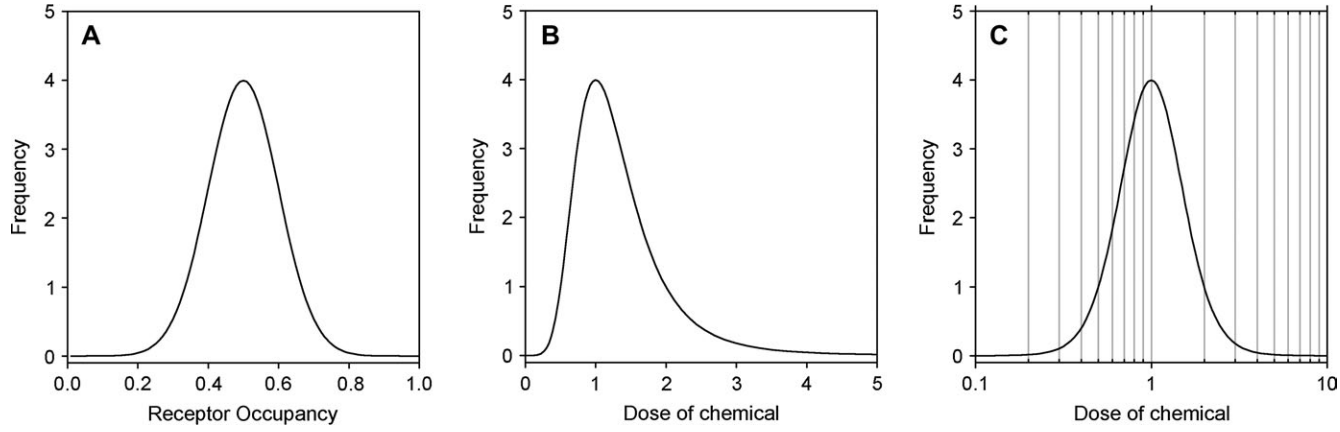

FIG. 3. Fractional dose-incidence relationships calculated for the PK–PD model based on receptor binding, using mean receptor occupancy of $mu = 0.5$ and $sigma = 0.1$. Panel A is the incidence curve based on the BED (i.e., the fraction receptor occupancy); panels B and C show the frequency distribution plotted versus administered dose, with arithmetic and logarithmic dose scaling, respectively.

best, an indirect measure of the more important variable, i.e., the relevant BED at target tissues. The relationship between BED and administered dose for any chemical depends primarily on PK and PD characteristics of specific compounds and the mode of action by which compounds exert their biological effects. Thus, this overall relationship is expected to vary considerably among different compounds. In the face of this variability, the log normal distribution has been unaccountably successful in describing incidence of toxic responses (see Gaddum, 1945).

Koch discussed the mechanisms that could generate logarithmic distributions in various biological contexts (Koch, 1966). One was the case where a toxicant caused an effect when a minimal concentration was maintained for a certain period of time. If either the elimination constant or the minimum required time period above the minimum concentration (but not both) were normally distributed in the population, a log normal distribution would be expected. This earlier exercise had motivations similar to the PK–PD modeling simulations developed in the present paper. Our work has shown that a log normal distribution might develop even though the underlying response of the tissue to toxicant was not itself defined by a log normal distribution. In the case of Koch's example, the toxicity was assumed to occur under equivalent conditions in all individuals: variability in kinetic parameters give rise to differences in susceptibility among individuals.

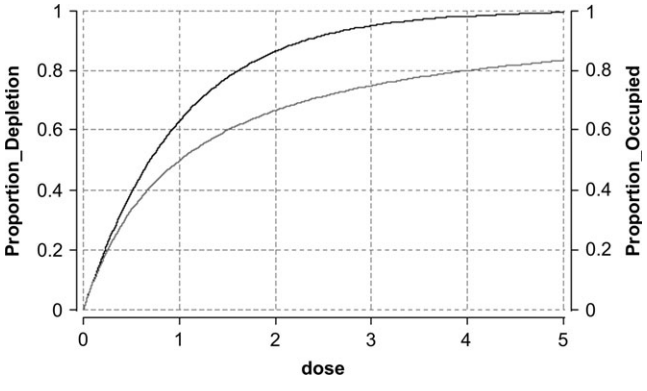

FIG. 4. Nonlinear relationships between BED and administered dose for reactivity and receptor occupancy modes of action. In each type model examined, the dose-BED curve starts at 0.0, and asymptotically approaches 1.0 as dose increases.

Creating Quantal Dose—Response Relationships from Continuous Measures

Our examples with reactivity (this paper) and receptor occupancy (Lutz *et al*., 2005) convert continuous measures of BED to quantal (binary) measures of incidence. We are not aware of other studies that have examined dose-incidence relationships through PK–PD modeling by linking BEDs with incidence through tolerance distributions. An earlier PD modeling study simulated chloroform toxicity by relating rates of metabolism and probability of cell death. Reitz between *et al*. (1990) developed the computational model for chloroform cytotoxicity that included a linkage between cell killing and the rate of metabolism of chloroform. The link was provided by using a distribution for the maximum rate of repair in a population of cells. This earlier work has some similarities to our examination of distributions for toxic responses. The sensitivity of individual cells was described as normally distributed with a mean and standard deviation of a maximum repair rate. For any rate of chloroform metabolism some portion of cells, i.e., those with repair rates lower than the rate of metabolism to reactive metabolites, were at risk for cell death, This distribution of normal distribution of the repair rate combined with the dose dependent rate of metabolism provided the relationship of BED with cell killing by chloroform. No efforts were made with this chloroform model to estimate whether a dose-incidence curve for this mode of action would

lead to a log normal relationship between incidence and administered dose.

In two papers (Bogen, 1990; Bogen and Gold, 1997) carcinogenic responses of several halogenated hydrocarbons were linked to measures of the metabolized dose. Cancer dose-incidence curves versus these measures of metabolized dose were simply assumed to be represented by a log normal relationship with values for *mu* and *sigma*. These distributional parameters were fit to cancer incidence data and then the fitted distributions were used to predict cancer incidence for various exposure scenarios. The approach with these halogenated compounds was not intended, as with our work, as an attempt to examine the nature of the expected relationship between incidence and tissue dose. In addition, the fitting with these compounds was based on a measure of tissue dose, without consideration of any relationship between the tissue dose and a BED.

Models for Tissue Reactivity as a Mode of Action

Here, we assumed a normal susceptibility distribution between BED and incidence and found that the nonlinearity for the relationship between administered dose and BED alone can be sufficient to generate a log normal curve for the relationship between administered dose and incidence. The ability of this procedure to recapitulate log normal distributions for reactivity and for receptor occupancy modes of action indicates a broader applicability of our results. However, the PK models used to date for examining this question are highly simplified. In the future, other examples could be examined by computer simulation of more complex PK processes rather than using situations that give rise to simple algebraic relationships to represent the relationship of BED and dose. Simulation models, based on more complete PBPK models, could be developed to explore different modes of toxicity, more varied dosing regimens, and might even include variability of model parameters. However, these simulation models would still explore the same question: do log normal relationships for administered dose arise from normal relationships for incidence with BED, confounded by nonlinear relationships between administered dose and BED?

PD Models—Proportionate Responses versus Tolerance Distributions

A PBPK model was used to assist in conduct of a cancer risk assessment for vinyl chloride. The BED in this model was the liver exposure to a reactive intermediate, estimated as daily amount of vinyl chloride metabolized to an oxirane intermediate per unit volume of liver (Clewell *et al*., 2001). In this approach to cancer risk assessment, the probability of tumor was related directly to this BED. A similar type of PK–PD model-based risk assessment is illustrated by recent work with formaldehyde (Conolly *et al*., 2003). Two measures of BED with this compound considered to be proportional to mutation rate, i.e., DNA–protein cross-links and cell proliferation, were measured directly. The probability of tumors at any inhaled concentration was estimated using a two-stage cancer model in which cell birth, cell death, and mutation were treated as stochastic processes. The predicted outcome from the model represents probability of response at a specified concentration in a uniform population. These PK–PD modeling approaches do not invoke a distribution of sensitivities for a diverse group of individuals (or cells) in a population. They do, however, base the probability of responses on measures of BEDs.

In these two examples, the response endpoint was cancer, although the formaldehyde incidence was heavily influenced by cytotoxicity, cell death, and regenerative cell proliferation. Our modeling was developed to consider a wider array of cellular processes, including cytotoxicity and receptor-mediated replication where a good portion of the reserves of the cell have to be overcome to initiate toxicity or where a high degree of occupancy has to be achieved to initiate processes leading to cell division. With the use of the tolerance distribution, the underlying tissue response more resembles a threshold for any individual; there is no response up to a certain dose, then a response above a critical dose. Is this depiction a realistic picture of cellular response to toxicity or proliferation? Increasingly, the responses of cells to stressors appear to be binary in nature, i.e., all-or-none rather than a graded response. This pattern is obviously true for responses such as cell death or cell division; however, all-or-none responses at a cellular level also appear valid for induction of networks of genes and of single genes (Louis and Becksei, 2002). These cellular switches (all-or-none behaviors) are frequently governed by genetic networks involving autocatalytic mitogen activated protein kinases (MAPKs) or other nonlinear positive feedback processes within cells (Bhalla *et al*., 2002; Ferrell, 2002). The advent of high throughput genomics and computational approaches that will allow reconstructing and modeling cell signaling networks and network perturbations by toxic compounds should permit mechanistic evaluations of response thresholds and a better mechanistic understanding of tolerance distributions for toxic responses (Andersen *et al*., 2005).

Summary

In developing PD models for incidence of toxic responses associated with loss (or alteration) of biological functions due to tissue reactivity or receptor occupancy, tolerance distributions for incidence of responses in a population should be assumed to be normally distributed in relation to appropriate measures of BED. This conclusion, based on the modeling approaches in this paper, is consistent with chemical and biological considerations about modes of action for toxic responses by chemical exposures and still approximates log normal distributions in relation to administered dose for most situations.

APPENDIX A

The normal distribution for reactivity has a BED related to proportion of the macromolecule depleted (MMd) with a mean depletion (*mu*) and a standard deviation (*sigma*). Using Equation 7, with normalization to the composite parameter value of 1.0, allows calculation of dose and incidence for any presumed level of depletion from 0.0 to 1.0. The PK–PD models were written in ACSL (Aegis Technologies, Austin, TX) using the proportion macromolecule depleted as the independent variable. The reactivity model from Equations 1–7 was then condensed into very simple code with the two equations below. MMinit would be the initial value to the proportion of unreacted target, i.e., 1.00.

$$\text{var } iable\_MMd = 0.01 \text{ (ranges from 0.00 to 1.00)}$$

$$\begin{aligned} dose &= \log\{MMinit/(MMinit - MMd)\} \\ &= \log/\{1/(1.0 - MMd)\} \end{aligned}$$

$$\begin{aligned} Incidence = \{1/\sqrt{2\pi * sigma\_(MMd)}\} \\ *\exp\{-(MMd - mu)^2/2 * sigma^2\} \end{aligned}$$

ACKNOWLEDGMENTS

This work was supported by the Long Range Research Initiative (LRI) of the American Chemistry Council (ACC) to MEA.

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