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FORUM

Dose-Incidence Relationships Derived from Superposition of Distributions of Individual Susceptibility on Mechanism-Based Dose Responses for Biological Effects

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Dose-response relationships for incidence are based on quantal response measures. A defined effect is either present or not present in an individual. The dose-incidence curve therefore reflects differences in individual susceptibility (the "tolerance distribution"). At low dose, only the more susceptible individuals manifest the effect, while higher doses are required for more resistant individuals to be recruited into the affected fraction of the group. Here, we analyze how such dose-incidence relationships are related to mechanism-based dose-response relationships for biological effects described on a continuous scale. As an example, we use the quantal effect "cell division" triggered by occupancy of growth factor receptors (R) by a hormone or mitogenic ligand (L). The biologically effective dose (BED) is receptor occupancy (RL). The dose-BED relationship is described by the hyperbolic Michaelis–Menten function, $RL/R_{tot} = L / (L + K_D)$. For the conversion of the dose-BED relationship to a dose–cell division relationship, the dose-BED curve has to be combined with a function that describes the distribution of susceptibilities among the cells to be triggered into mitosis. We assumed a symmetrical sigmoid curve for this function, approximated by a truncated normal distribution. Because of the supralinear dose-BED relationship due to the asymptotic saturation of the Michaelis–Menten function, the composite curve that describes cell division (incidence) as a function of dose becomes skewed to the right. Logarithmic transformation of the dose axis reverses this skewing and provides a nearly perfect fit to a normal distribution in the central 95% incidence range. This observation may explain why dose-incidence relationships can often be described by a cumulative normal curve using the logarithm of the administered dose. The dominant role of the tolerance distribution for dose-incidence relationships is also illustrated with the example of a linear dose-BED relationship, using adducts to protein or DNA as the BED. Superimposed by a sigmoid distribution of individual susceptibilities, a sigmoid dose-incidence curve results. Linearity is no longer observed. We conclude that differences in susceptibility should always be considered for toxicological risk assessment and extrapolation to low dose.

Key Words: **dose-response relationship; models; logarithm; tolerance distribution.**

Textbooks of pharmacology and toxicology describe two types of dose-response relationships (DRR), one for (1) the relationship between the concentration of a drug or toxicant and the intensity or strength of an effect, and another for (2) the relationship between a dose and an incidence of a defined effect in a group (Hardman and Limbird, 2001; Klaassen, 2001). By plotting concentrations or dose on a logarithmic scale, data from both types become sigmoid. This may explain why fundamental differences between the two types of DRR are often overlooked. Differences refer to the quality of the response variable on the y-axis, the question about the driving force for the sigmoid shape of the dose-response curves, and the consequences for extrapolation to low dose.

In the first type DRR, the response variable usually is a continuous measure (a "biologically effective dose" BED), for instance, a concentration of a biomarker such as the concentration of a receptor-ligand complex (RL). The relationship between the concentration of ligand L and BED is described by the Michaelis–Menten function $RL/R_{tot} = L/(L + K_D)$, which starts at point (0, 0) and bends asymptotically to $y = 1$. Logarithmic scaling of the dose scale stretches the low-dose part of the curve to minus infinity so that the curve becomes sigmoid simply by data transformation.

The second type DRR (dose-incidence relationship) is based on a quantal (binary) measure of response, i.e., data points represent proportions of individuals in a population that show a defined effect within a defined period of observation. Here, the distribution of susceptibilities in the given population (also defined as "tolerance distribution") determines the shape of dose-incidence curve.

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In toxicological risk assessment, the fundamental difference between these two types of dose-response relationship is not always recognized, so that extrapolation of incidence data is often discussed on the basis of dose-BED relationships alone. For instance, cancer incidence from exposure to a DNA-reactive carcinogen is often considered to be linear with low dose, based on the mechanistic hypothesis that the rate of DNA adduct formation is proportional to dose at the low dose end. This does not take into account differences between individuals for the steps that follow DNA adduct formation. Going directly from the continuous dose metrics ''levels of DNA adducts'' to the quantal dose metrics ''cancer incidence'' without consideration of individual susceptibilities is unlikely to provide a correct estimate of the risk at low dose.

Variability of humans for cancer risk has been introduced as individual time-to-tumor 30 years ago (Albert and Altshuler, 1976). Statistical aspects (Haseman and Hoel, 1979) and pharmacokinetic considerations (Hattis *et al.*, 1987) followed. Differences in individual susceptibility have also been discussed for the estimation of the proportion of animals outside a normal range of a biologically important variable (Gaylor and Slikker, 1990), for risks at low dose (Gaylor and Kodell, 2002), and for the concept of ''critical effect size'' when a quantal response (e.g., fraction of animals with atrophy) is related to the underlying continuous response, such as degree of atrophy (Slob, 1999).

Here, we show how a dose-incidence curve can be generated from a dose-BED relationship. The procedure is exemplified for cell division triggered by receptor occupancy as a function of ligand concentration (Andersen *et al.*, 2002). Quantification includes fitting the composite dose-incidence curves with different models. In this context, we address the question why a logarithmic scaling of the dose axis often results in a good fit by a normal distribution. The dominant role of the susceptibility distribution in determining shapes of dose-incidence curves will then be illustrated with an example of a linear dose-BED relationship, such as the formation of macromolecular adducts. Parts of our analysis include generally accepted facts or have been mentioned in other contexts, but we are not aware of any publication where these issues had been presented in the general manner illustrated and exemplified here with the focus on applicability to toxicology and risk assessment.

METHODS AND PROCEDURES

From ligand concentration to receptor occupancy. Receptor occupancy is a nonlinear (hyperbolic) function of local concentration of ligand, as defined by

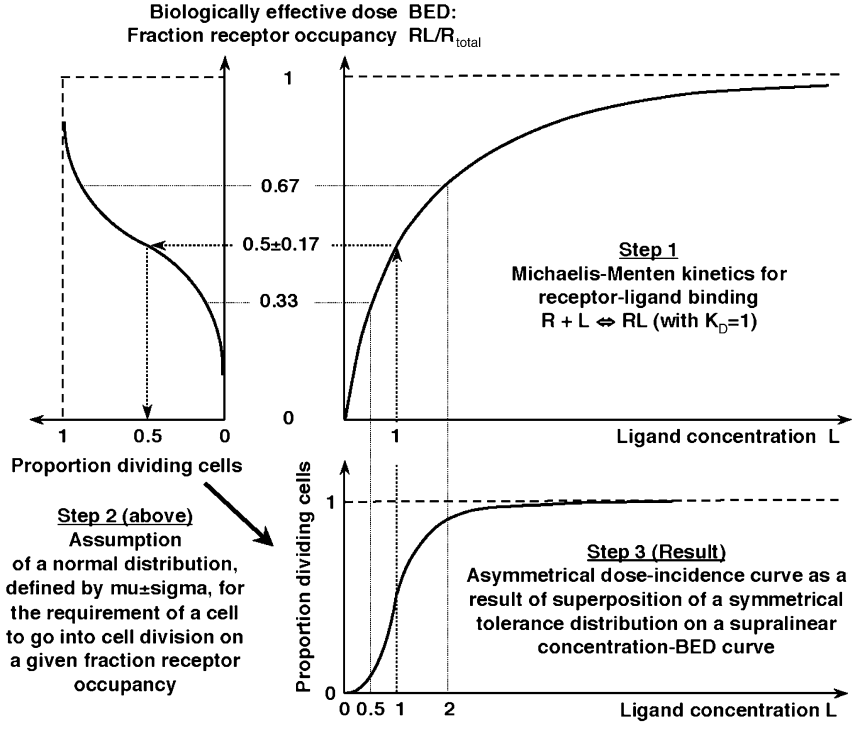

FIG. 1. Schematic illustration of the generation of a dose-incidence curve (bottom right-hand panel) from a relationship between a concentration of a drug or toxicant and a continuous-effect measure (the biologically effective dose, BED), by superposition of the dose-BED curve (top right panel) with a susceptibility distribution. Assumptions are growth receptor–ligand interaction for step 1, superimposed by the symmetrical cumulative normal distribution for cells to initiate cell division (step 2). Step 3 shows the resulting dose-incidence relationship, which is skewed to the right because of the nonlinear (''supralinear,'' ''saturating'') shape of ligand concentration–BED relationship.

Michaelis–Menten binding kinetics, $RL/R_{tot} = L/(L + K_D)$, with R, L, and RL designating the concentration of receptor, ligand, and receptor-ligand complex, respectively. K_D is the dissociation constant; RL/R_{tot} represents the biologically effective dose BED and is the fraction receptor occupancy. It is a continuous measure with values between 0 and 1. The hyperbolic relationship between RL/R_{tot} and L is shown in the top right-hand panel of Figure 1.

Susceptibility of cells to go into division. The minimum BED required for a cell in an organ to go into division was assumed to follow a normal distribution (step 2), which is described by mean *mu* and standard deviation *sigma.* For the example shown in Figure 1 (top left-hand panel), we chose $mu = 0.5$ and $sigma = 0.17$.

Superposition of the two functions. Superposition of the susceptibility distribution on the dose-BED curve results in the dose-incidence curve that follows:

$$\Phi(((L/(L+K_D)) - mu)/sigma)$$

where Φ is the cumulative standard normal distribution function

$$\Phi(x) = \int_{-\infty}^{x} \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{1}{2}t^2\right) dt$$

which assumes the values 0, 0.5, and 1 for $x = -\infty$, 0, and $+\infty$, respectively.

Fitting lognormal or normal distributions to the dose-incidence curves. For the fitting of a cumulative lognormal or a cumulative normal distribution to the calculated dose-incidence curves (result of step 3 in Fig. 1), means *m* and standard deviations *s* of $\Phi((\ln(L) - m)/s)$ or $\Phi((L - m)/s)$ were chosen. The central 95% incidence range ($0.025 < y < 0.975$) was divided up into one thousand equidistant dose segments, and the thousand incidence differences between the incidence curve and Φ were summed up. The result corresponds to an area, which was used as a measure of fit. The larger the subsumed area, the worse is the fit. This procedure overcomes differences in the scaling of the dose axis due to the use of different values for *mu* and *sigma.* Values of *m* and *s* were then searched for minimum area between the curves, for both lognormal and normal curves. This process was applied for a variety of combinations for *mu* and *sigma* (i.e., the means and standard deviations of the normal susceptibility distribution as shown in step 2 of Fig. 1), and provided the numbers listed in Table 1.

TABLE 1
Quantitative Analysis of Fit, Comparing Normal Distributions Fitted to Curves Using Logarithmic or Arithmetic Scaling of the X- (Dose) Axis

	sigma = 0.05		*sigma* = 0.10	
Dose scale	logarithmic	arithmetic	logarithmic	arithmetic
mu =				
0.20	13.91	9.52	24.12	17.93
0.25	9.91	10.15	17.74	19.74
0.30	7.06	10.87	12.67	21.30
0.35	4.85	11.70	8.51	22.91
0.40	3.01	12.66	4.94	24.70
0.45	1.37	13.79	2.27	26.77
0.50	0.50	15.14	2.15	29.18
0.55	1.72	16.77	4.53	32.01
0.60	3.38	18.80	7.91	35.33
0.65	5.25	21.36	11.74	39.20
0.70	7.52	24.70	16.29	43.69
0.75	10.46	29.18	21.94	49.60
0.80	14.58	35.33	28.41	59.78

Note. The true dose-incidence relationship resulted from superposition of a Michaelis–Menten curve with cumulative normal tolerance distributions defined by mean *mu* and standard deviation *sigma* (see top left panel of Fig. 1). Values indicate the level of discordance between the true curve and the best fits.

RESULTS

From a Dose-BED Curve to a Dose-Incidence Relationship

Figure 1 illustrates the procedure to generate a cumulative dose-incidence curve (bottom right panel) from a dose-BED curve (top right panel) after transformation with an assumed normal tolerance distribution (top left panel). The Michaelis–Menten function for growth receptor–ligand interaction was used to illustrate step 1. The curve starts at x,y-point (0, 0) and goes to $y = 1$ in an asymptotic manner with increasing ligand concentration. A Michaelis–Menten constant $K_D = 1$ unit of ligand concentration was assumed for a 50% fraction receptor occupancy.

Requirements of cells to go into cell division were assumed to be normally distributed with respect to fractional receptor occupancy, using mean $mu = 0.5$ and standard deviation $sigma = 0.17$ for this example (Fig. 1, top left panel). That is, for a cell with average susceptibility to be triggered into mitosis as a function of fraction receptor occupancy, 50% of the receptor molecules must carry a ligand in order for this cell to go into division. At 33% receptor occupancy (*mu – sigma*) about 16% of the cells will divide, at 67% receptor occupancy (*mu + sigma*) about 84% of the cells will be recruited into the effect, as given by the cumulative normal distribution. Based on $K_D = 1$, 33% receptor occupancy is achieved with ligand concentration 0.5 ($RL/R_{tot} = 0.5/(0.5 + 1) = 0.33$), 67% occupancy is achieved with ligand concentration 2 ($RL/R_{tot} = 2/(2 + 1) = 0.67$).

The dose-incidence curve in the bottom right panel of Figure 1 shows the result of the superposition of the tolerance distribution on the dose-BED curve. A right skew occurs due to the supralinear shape of the Michaelis–Menten function. Using a $\log_{10}$ transformation of the dose axis, the concentration values 0.5, 1, and 2 become −0.3, 0, and +0.3, i.e., the right skew is reversed to the symmetry of the normal susceptibility distribution. This result is not surprising, since the logarithmic form of the Michaelis–Menten function, $y = 10^x / (10^x + 1)$ represents a special case of the symmetrical logistic function. Our example therefore provides a conceptual explanation of why a *log*normal curve may sometimes provide a good fit even when the tolerance for BED is normally distributed.

Fitting Truncated Normal and Lognormal Curves to the Dose-Incidence Relationships

Figure 2 shows the best cumulative normal curves (dotted lines) or cumulative lognormal curves (dashed lines) that could be fitted to the curve generated by step 3 in Figure 1 (full line).

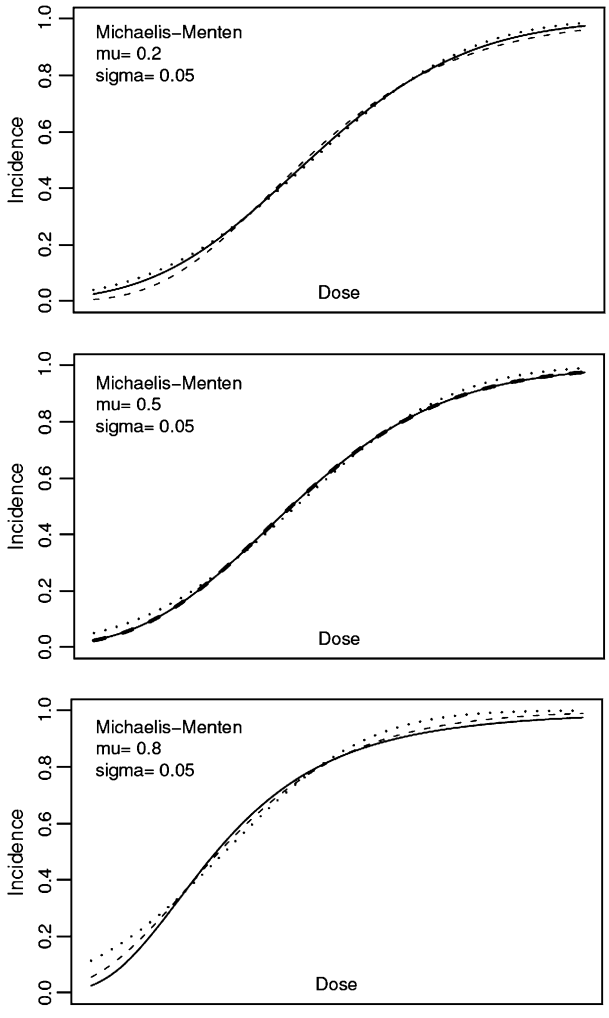

FIG. 2. Fitting a cumulative normal curve (dotted line) or a cumulative lognormal curve (dashed line) to the dose-incidence curve that resulted from superposition of a cumulative normal susceptibility distribution on a Michaelis–Menten curve (full line; the curve generated by step 3 in Fig. 1). The three panels show best fits for three different means of susceptibility (*mu* = 0.2, 0.5, and 0.8 receptor occupancy, respectively, for half of the cells to be triggered to divide), using the same standard deviation *sigma* = 0.05. Referring to Fig. 1, "Incidence" is proportion of dividing cells; "Dose" is ligand concentration.

Fitting was restricted to the central 95% incidence range, which excludes the asymptotic tails of the normal distribution. The three panels show the results for three choices of the mean susceptibility of cells to be triggered into division as a function of fraction receptor occupancy, *mu* = 0.2, 0.5, and 0.8. The standard deviation *sigma* was 0.05, $K_D = 1$. The solid line shows the incidence curve, the dotted line represents the best fit that can be achieved by a truncated normal distribution, and the dashed line is the best fit for a truncated lognormal distribution.

At low-level receptor occupancy (*mu* = 0.2; top panel), a normal distribution provided a slightly better fit than the lognormal. For *mu* = 0.5 (center panel), the best fit was achieved with a lognormal distribution; the overlap of the dashed and full lines for this case was almost perfect. For *mu* = 0.8 (bottom panel) logarithmic representation of the dose axis gave a better fit than using untransformed dose, although not as good a fit as at *mu* = 0.5.

For regulatory purposes, the low end of the distribution is most important. Figure 2 demonstrates that the lognormal fit underestimates the incidence at low doses when *mu* is relatively small compared to *sigma* (top panel). When *mu* is large relative to *sigma* (bottom panels), the lognormal distribution overestimates the incidence at low doses.

Numeric information on the fits is given in Table 1, for a range of *mu*-values (0.2 to 0.8) and for two standard deviations (*sigma* = 0.05 or 0.1). The values relate to areas between the dose-incidence curves for the two examples (the full lines in Fig. 2) and the respective best-fitting curves (dashed for best-fitting lognormal distribution, dotted for best-fitting normal distribution). The smaller the area, the better is the fit. The lognormal fit was better for all *mu*-values ≥ 0.25, with an optimum fit at *mu* = 0.5. The fit provided by the normal distribution had no optimum but deteriorated monotonically with increasing *mu.* These calculations indicate that responses triggered at high occupancy are more likely to be well described by lognormal distributions than responses triggered at low occupancy.

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 goodness of fit for both the logarithmic and arithmetic dose scale. The worsening was more pronounced for the arithmetic dose scale. This means 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. For all calculations, K_D 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.

Linear Dose-BED Relationship

Figure 3 shows what happens when a linear dose-BED relationship (top right panel) is superimposed by a sigmoid susceptibility distribution. The BED shown in the top right panel could, for instance, be the rate of covalent binding of a chemically reactive toxicant (or metabolite) to a biological macromolecule such as protein or DNA. For this reaction, the rate of adduct formation with concentration may be approximately proportional to the concentration of toxicant as long as enzymatic reactions involved are far below the Michaelis constant.

One of the consequences of adduct formation with protein could be cell death. Minor levels of protein damage would likely be tolerated and not result in cell death. At high damage levels, on the other hand, all cells would be killed. Therefore, the proportion of cells that become apoptotic or necrotic follows the respective tolerance distribution. We assumed

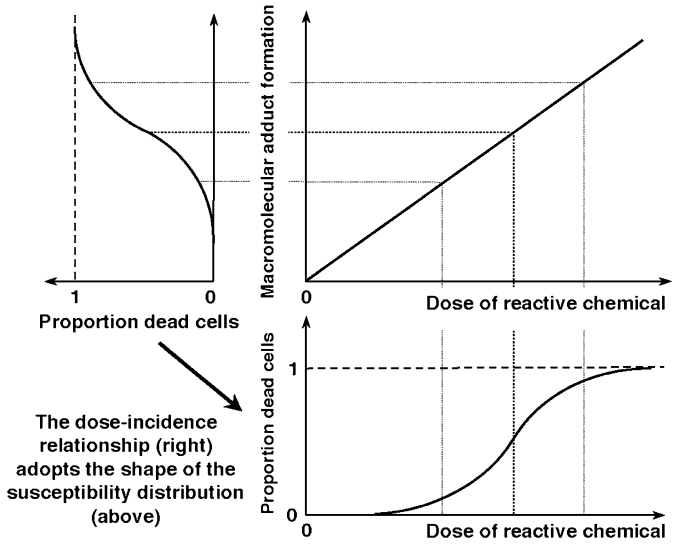

FIG. 3. Schematic illustration of the generation of a dose-incidence curve for proportion cell death (bottom right-hand panel) from a *linear* dose-BED relationship by superposition with a symmetrical susceptibility distribution for cells to die at a given adduct level. Assumptions are dose-proportional macromolecular adduct formation superimposed by a cumulative normal distribution for cell death. The bottom right panel shows the resulting *nonlinear* ''dose-incidence'' relationship.

a symmetrical sigmoid curve (top left panel of Fig. 3). The bottom right panel shows the result of the superposition. The dose-incidence curve is sigmoid, i.e., the (sigmoid) tolerance distribution was more important for the shape of the dose-incidence curve than the (linear) dose-BED curve.

DISCUSSION

We showed that (1) the shape of a dose-incidence curve is dominated by the distribution of tolerance of individuals in the given population and that (2) good fit of a cumulative normal distribution to incidence data plotted against log (dose) could be explained by the supralinear shape of the relationship between administered dose and biologically effective dose.

Sigmoid Tolerance Distributions

Sigmoid shapes of tolerance distributions have been used for decades, based primarily on empirical observations and the central limit theorem. In its simplest form, this mathematical law states that the sum of many (r) independent, identically distributed random variables is, in the limit as $r \to \infty$, normally distributed.

For complex reactions of a cell or organism to a drug or toxicant, or for multistage requirements as postulated for carcinogenesis, the rate of the process is modulated by a number of factors, all of which contribute to the individual susceptibility. For instance, the risk of cancer after exposure to an activation-dependent DNA-reactive carcinogen depends on the activity of enzymes associated with metabolic activation, detoxification, and DNA repair, on cell cycle checkpoints, rates of cell division and apoptosis, as well as on immune responses to eliminate premalignant cells (Lutz, 2001). The central limit theorem provides a theoretical basis of using normal distributions. However, in biological systems, the number of variables is finite, and biological boundaries are expected to limit the span of individual susceptibilities. If an individual is ''on the bad side'' for too many susceptibility factors, it might no longer be capable of life. For the model function, this is equivalent to truncation. The observable tolerance distribution would still be approximately normal, but the problem of incidences at negative doses associated with a full normal distribution has been overcome.

Normal Distributions after Logarithmic Transformation of the Dose Axis

Dose-incidence relationships are often well described by cumulative *"lognormal"* curves, i.e., by cumulative normal curves with dose plotted on a logarithmic scale. A number of explanations have been put forward to explain this phenomenon. According to the central limit theorem of statistics, the distribution will be *log*normal for the *product* (instead of *sum*) of the variables or when the error in measurement is proportional to the value of the measurement (Limpert *et al.*, 2001). The question whether multiplicative combination of factors that contribute to susceptibility reflects biology better than additive combination is an aspect worth further thought.

Other attempts to explain the success of logarithmic dose transformations included time factors. Koch published two papers on the logarithm in biology, giving examples of how the logarithm comes into play in an exact form (Koch, 1966), or as an approximation (Koch, 1969). For the exact solution, he considered a toxicant that was excreted with a first-order rate constant, k, and caused an effect when a minimal concentration was maintained for a certain period of time. Under these conditions, if either the elimination constant or the minimum required time period (but not both) is normally distributed in the population, a lognormal distribution for dose results.

An early paper that used lognormal distributions for cancer risk estimation was published by Mantel and Bryan (1961). A major problem was the estimation of the probit slope which is, in fact, a measure of the span of individual susceptibilities. Lognormal distributions of variables have been reviewed not only for different health risks (Hattis *et al.*, 1999; Hattis and Silver, 1994) but also for other disciplines, such as geology and mining, ecology, or food technology (Limpert *et al.*, 2001).

Here, we provided a simple explanation for the logarithm, which requires neither multiple variables nor time factors. It is based on the fact that many biologically effective doses show saturation with administered dose, i.e., exhibit a supralinear shape at the high-dose end. This behavior results in a right skew

after superposition with a symmetrical tolerance distribution, which can brought back to approximate symmetry by using a logarithmic dose scaling. The example here was based on receptor-ligand interaction; another example is presented in the companion article (Andersen *et al*., in press).

Implications for Risk Assessment

Our examples show how a continuous response measure can be translated to a quantal (binary) measure. This conversion is key to understanding dose-incidence relationships in pharmacology and toxicology. While susceptibility distributions dominate the shape of dose-incidence curves, mechanistic considerations are still important. They not only determine the shape of the dose-response curve of step 1 in Figure 1, but they also give leads to critical factors that modulate susceptibility. Low-dose extrapolation in risk assessment must include aspects of individual susceptibility. The question about incidence at low dose is in fact equivalent to the question about ''who is the most susceptible individual?'' (Lutz, 2002). For prevention, it might therefore be particularly rewarding to reduce exposure in those individuals that are most susceptible and to counteract the factors that are associated with high susceptibility. As taken from the title of a publication by Dale Hattis (1996), ''Human interindividual variability in susceptibility to toxic effects [should no longer be] an annoying detail but a central determinant of risk.''

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