# Quantifying Relative Potency in Dose-Response Studies

**Gregg E. Dinse and David M. Umbach**

**Abstract** Relative potency is an important concept in the comparative evaluation of chemicals via dose-response studies. For example, toxicologists use relative potency estimates to rank chemicals with respect to a given response endpoint, to convert doses of one chemical to equivalent doses of another chemical, and to combine information across studies and endpoints when calculating toxic equivalency factors. The conventional definition of relative potency, arising historically from dilution assays, is a ratio of equi-effective doses, that is, those doses that produce the same mean response. Specifically, the ratio is the dose of a reference chemical divided by the dose of a test chemical. In an analytical dilution assay, relative potency is constant regardless of the mean response used to select equi-effective doses. Nevertheless, researchers often observed data that were inconsistent with constant relative potency and desired ways to characterize non-constant relative potency. This article reviews various approaches for quantifying relative potency when it cannot be regarded as constant, including modifications to the usual definition. In particular, we focus on recent proposals that describe the relative potency of two chemicals as functions of dose or of response.

## Introduction

Relative potency plays a critical role in toxicology. For example, toxicologists estimate relative potency to rank chemicals with respect to a toxicity endpoint of interest (e.g., [1]), to convert a dose of one chemical to an equivalent dose of another chemical (e.g., [2]), and to combine information across studies and endpoints when calculating a chemical's toxic equivalency factor (e.g., [3]). Relative potency is

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typically derived from the parameters in a mathematical (dose-response) model that expresses a toxicity response as a function of a chemical dose.

Consider a dose-response function that relates the mean response for a particular endpoint to the dose of a given chemical. Let $f(d;\theta)$ be a model that specifies mean response in terms of dose $d$ and parameter vector $\theta$. We focus on models for which $f$ is a monotone increasing function of $d$, though the same methods can be modified easily to handle monotone decreasing dose-response functions. Early methods for comparative bioassays often assumed a linear model for $f$, possibly after transforming dose, response, or both. Often, linearity is reasonable over some restricted dose-response region only. A linear dose-response model specifies $f(d;\theta) = \alpha + \beta d^*$, where $\theta = (\alpha, \beta)$, $\alpha$ is an intercept, $\beta$ is a slope, and $d^*$ is a dose metric (typically either dose itself or log dose). Other assays, especially those for binary endpoints, frequently employed a sigmoid model with lower and upper response asymptotes and expressed generally as:

$$f(d;\theta) = L + (U - L)\, g(d;\phi), \tag{1}$$

where $L$ is the lower response limit, $U$ is the upper response limit, and the dose-quantile function $g$ is a monotone increasing function of $d$ that ranges from 0 (at $d = 0$) to 1 (at $d = \infty$) and depends on a parameter vector $\phi$, with $\theta = (L, U, \phi)$. If mean response decreases as dose increases, we associate $U$ with $d = 0$ and $L$ with $d = \infty$ and require $g$ to be monotone decreasing in dose. In either case, the elements of $\phi$ typically govern the location and shape of the dose-response curve.

Now consider multiple chemicals. Without loss of generality, we focus on two chemicals: a reference chemical, $C_0$, and a test chemical, $C_1$. Rooted in ideas from dilution assays, relative potency, denoted $\rho$, is classically defined as the ratio of equi-effective doses (reference divided by test), i.e., doses of the two chemicals that elicit the same response. Ideally, in dilution assays, this ratio does not change with the response level chosen. Faced with examples where the ratio did vary with response level, investigators had to grapple with ways to characterize non-constant relative potency.

This article reviews approaches that have been proposed for assessing non-constant relative potency. Some of these retain the classical definition of relative potency as a ratio of equi-effective doses but abandon the notion that a single numerical constant suffices to compare potency of two chemicals. Others retain the simplicity of a single constant to compare potency between chemicals but abandon or modify the classical definition. The most recent developments describe non-constant relative potency using the notation of mathematical functions.

## Constant Relative Potency in Bioassay

The classical concept of relative potency arises from analytical dilution assays, where each test preparation is constructed as a dilution of a reference preparation [4]. In this context, relative potency as the ratio of equi-effective doses is a

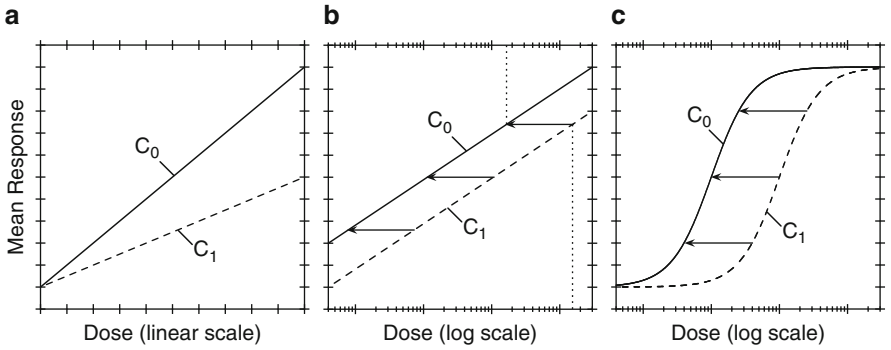


**Fig. 1** Dose-response curves producing constant relative potency. Panels: (**a**) diverging lines with equal intercepts, where mean response is linear in dose; (**b**) parallel lines with equal slopes, where mean response is linear in log dose; and (**c**) similar sigmoid curves, generated by Hill functions with equal response limits and shapes. In all three panels, the ratio of any equi-effective doses for reference chemical $C_0$ and test chemical $C_1$ is constant and equals the relative potency. In panels (**b**) and (**c**), the length of each horizontal arrow from $C_1$ to $C_0$ is constant and equals the log relative potency. In panel (**b**), the vertical dotted lines illustrate that a given arrow (or relative potency) can be indexed by the dose of either chemical, as well as by mean response

constant, $\rho$, regardless of the response level considered. When relative potency is constant, ranking chemicals is straightforward: simply rank them by the relative potencies. Dose conversion is also simple: the dose of chemical $C_0$ that is equivalent to dose $d_1$ of chemical $C_1$ is $d_0 = d_1\rho$, and the dose of $C_1$ that is equivalent to dose $d_0$ of $C_0$ is $d_1 = d_0/\rho$. Furthermore, because the ratio of equi-effective doses is constant, the difference between the logs of those doses is also constant. Thus, as often noted, relative potency is constant if and only if the dose-response functions are identical except for a horizontal shift when plotted against log dose (though this graphical definition can be inconvenient when zero doses are involved). When the relative potency of two chemicals is constant, their dose-response curves are referred to as similar.

## *Slope Ratio Assays*

A slope ratio assay is based on dose-response curves that are linear functions of dose with a common intercept (usually the origin) but possibly distinct slopes [4]. Thus, the dose-response function for $C_i$ is $f(d;\theta_i) = \alpha + \beta_i d$, where $\beta_i > 0$ and $\theta_i = (\alpha, \beta_i)$ for $i = 0,1$ (Fig. 1a). Denoting the dose of $C_i$ that produces mean response $\mu$ by $d_i(\mu)$, the corresponding inverse function for $C_i$ is $d_i(\mu) = f^{-1}(\mu;\theta_i) = (\mu - \alpha)/\beta_i$ and relative potency is a constant ratio of the slopes: $d_0(\mu)/d_1(\mu) = \beta_1/\beta_0$ for all values of $\mu$.

### *Parallel Line Assays*

A parallel line assay is based on dose-response curves that are linear functions of log dose with a common slope but possibly distinct intercepts [4]. Thus, the dose-response function for $C_i$ is $f(d;\theta_i) = \alpha_i + \beta\log(d)$, where $\beta > 0$ and $\theta_i = (\alpha_i, \beta)$ for $i = 0,1$ (Fig. 1b). The corresponding inverse function is $d_i(\mu) = f^{-1}(\mu;\theta_i) = \exp[(\mu - \alpha_i)/\beta]$ and relative potency is again constant: $d_0(\mu)/d_1(\mu) = \exp[(\alpha_1 - \alpha_0)/\beta]$ for all values of $\mu$.

### *Assays Involving Similar Sigmoid Curves*

Consider chemicals that have sigmoid dose-response functions of the form given in Eq. 1. Suppose $L$ and $U$ are the same for both chemicals and that the vector $\phi$ is the same for both chemicals up to a location parameter for log dose. Then, the dose-response curves are similar, and the chemicals have constant relative potency. The Hill [5] model is an example. It is obtained by setting $g(d;\phi) = d^S/(d^S + M^S)$, where $S$ is a shape parameter and $M$ is the median effective dose ($ED_{50}$), which is the dose producing a mean response halfway between $L$ and $U$. Similar Hill curves have identical response limits and shapes; only their $ED_{50}$s differ (Fig. 1c). The corresponding inverse function for $C_i$ is $d_i(\mu) = f^{-1}(\mu;\theta_i) = M_i[(\mu - L)/(U - \mu)]^{1/S}$ and relative potency is a constant equal to the $ED_{50}$ ratio: $d_0(\mu)/d_1(\mu) = M_0/M_1$ for all values of $\mu$ between $L$ and $U$. The Hill model can be rewritten in its log logistic form by setting $g(d;\phi) = 1/[1 + \exp(-X)]$ with $X = S[\log(d) - \log(M)]$. Here, $g(d;\phi)$ is a logistic distribution function for $\log(d)$ with location parameter $\log(M)$ and scale parameter $1/S$ [6]. Analogously, the probit model takes the dose-quantile function $g(d;\phi)$ as the standard normal distribution function evaluated at $X$ [6]. Other distribution functions, such as the Weibull [7], can be used for $g(d;\phi)$, and $\phi$ can contain more than two parameters [8]. In any of these cases, similar sigmoid curves (and thus constant relative potencies) are obtained by constraining the dose-response models for $C_0$ and $C_1$ to be identical except for the location parameter.

## Non-constant Relative Potency

In many situations, the notion of constant relative potency is inconsistent with observed data, and investigators face a dilemma. One strategy is to retain the simplicity of a single constant as a descriptor of relative potency, even though treating relative potency as fixed when it is not can generate misleading conclusions [9]. This strategy can involve modifying or abandoning the classical definition of relative potency based on a ratio of equi-effective doses. An alternate strategy is to adopt a descriptor of relative potency that involves more than a single constant,

but this alternative has the undesirable side effect of making dose conversion or chemical ranking problematic. Despite an awareness that many pairs of chemicals have non-constant relative potency, few general approaches for handling non-constant relative potencies were developed until recently.

## *Defining Relative Potency as the Ratio of $ED_{50}$s*

Because similar sigmoid dose-response functions have constant relative potency given by the ratio of their $ED_{50}$s, some authors have simply employed that ratio as a measure of relative potency even for data where dose-response curves in log dose may differ by more than a constant horizontal shift (e.g., [10]). Others have pointed out that this approach is simple and convenient but less than ideal theoretically [11]. The convenience arises because an estimate of the $ED_{50}$ is usually output by software for fitting dose-response models. On the other hand, because this approach treats relative potency as constant despite evidence to the contrary, it can lead to flawed conclusions when ranking chemicals [9] and would certainly distort dose conversions.

A more subtle issue also arises. When two sigmoid curves have the same upper and lower response limits, the $ED_{50}$ values for each curve correspond to the same value of mean response for both curves. In that case, the ratio of $ED_{50}$s meets the classical definition of relative potency, at least at the single chosen response level. On the other hand, when the two curves differ in their upper and/or lower response limits, the $ED_{50}$ values for each curve typically correspond to distinct values of mean response for each curve and the classical definition of relative potency is lost. The doses are no longer equi-effective in the sense of having the same mean response; the doses instead mark the same proportional change in mean response between the respective lower and upper limits for each chemical.

## *Deforming the Log-Dose and Response Axes to Achieve Similarity via Splines*

Guardabasso et al. [12] proposed to fit the reference chemical's dose-response curve using a cubic spline function of log dose and then obtain the test chemical's dose-response curve by horizontally shifting and stretching the reference chemical's spline by constant amounts along the log dose axis – essentially deforming the log dose axis with a two-parameter transformation. They assumed that both chemicals had the same response limits and equated log relative potency with the constant shift parameter, even if the stretch (i.e., scale) parameter differed from 1. Thus, even though they reported a constant value that they called 'relative potency', they invoked an unconventional definition by allowing the dose-response curves to differ

by more than a constant horizontal shift along the log dose axis. Later, Guardabasso et al. [13] extended this approach to accommodate chemicals with different response limits by also allowing vertical shifting and stretching of the reference spline along the response axis. Their methods retained the simplicity of characterizing relative potency by a single parameter at the expense of redefining relative potency in a way that no longer matched the classical definition. Although the construction is a clever one, the utility of this approach for the traditional uses of relative potency, such as chemical ranking or dose conversion, seems questionable.

### *Evaluating Relative Potency at Multiple $ED_{100\pi}$ Values*

We have already mentioned the common approach of using the ratio of $ED_{50}$s to assess relative potency even if the dose-response curves are not similar. Of course, with non-constant relative potency, the $ED_{50}$ ratio can differ greatly from the $ED_{10}$ ratio, the $ED_{75}$ ratio, or any other ratio of $ED_{100\pi}$ values (for any $0 < \pi < 1$). One slight improvement on estimating non-constant relative potency by a single $ED_{100\pi}$ ratio would be to report several ratios [14] or the range between two effective doses, such as the $ED_{20}$ and the $ED_{80}$ [15]. Insofar as these proposals rely on $ED_{100\pi}$ values, as mentioned earlier, they entail a modification of the classical definition of relative potency when the two chemicals differ in their lower and/or upper response limits.

## Relative Potency Functions

From evaluating relative potency at a finite list of equi-effective dose levels, it is a short step to evaluating relative potency at every relevant dose level, that is, to defining a relative potency function.

### *Parallel Line Assays Where Similarity Fails*

Cornfield [16] derived a relative potency function under separate linear log-dose-response models. Assume that the mean response to dose $d_i$ of $C_i$ is $f(d_i;\theta_i) = \alpha_i + \beta_i \log(d_i)$ and $\theta_i = (\alpha_i, \beta_i)$ for $i = 0,1$ (for similarity, the slopes would be equal). The corresponding inverse function is $d_i(\mu) = f^{-1}(\mu;\theta_i) = \exp[(\mu - \alpha_i)/\beta_i]$, which allowed Cornfield to express log relative potency as a linear function of mean response $\mu$:

$$\lambda_\mu(\mu) = \log\left[\rho_\mu(\mu)\right] = \log\left(\frac{d_0(\mu)}{d_1(\mu)}\right) = \left(\frac{\alpha_1}{\beta_1} - \frac{\alpha_0}{\beta_0}\right) + \left(\frac{1}{\beta_0} - \frac{1}{\beta_1}\right)\mu. \qquad (2)$$

Here, the notation $\rho_{\mu}(\mu)$ denotes a relative potency function that maps $\mu$ to the relative potency at response level $\mu$. Cornfield noted that relative potency also can be indexed by the dose of either chemical (Fig. 1b) and derived formulae for $\rho_{d1}(d_1)$ and $\rho_{d0}(d_0)$ that express relative potency as functions of the doses of the test and reference chemicals, respectively. All three relative potency functions reduce to the constant obtained under the parallel line model if $\beta_0 = \beta_1 = \beta$. Cornfield's approach, which assumes a separate linear model in log dose for each dose-response curve, produces relative potency functions that are log-linear either in mean response or in log dose. His approach would be effective whenever a suitable transformation of the response yields a pair of dose-response models that are linear in log dose.

## *Specifying a Relative Potency Function a Priori*

DeVito et al. [17] addressed the problem of estimating relative potency when data on the reference chemical are adequate to fit a non-linear (i.e., Hill) dose-response model, but data on the test chemical are not. For example, when fitting a sigmoid, if responses at the highest tested doses do not level out, estimation of the upper response limit (and thus the $ED_{50}$) becomes problematic. DeVito et al. [17] proposed the following ad hoc solution: (i) fit a Hill model to the reference chemical data; (ii) invert this Hill model to express dose as a function of mean response; (iii) for each (dose-specific) sample mean response in the test group, apply the inverse model to predict an equivalent dose of the reference chemical (say $\hat{d}_0$); and (iv) fit a linear model for equivalent reference dose in terms of actual test dose (say $d_1$) to give: $\hat{d}_0 = \alpha + \beta d_1$. If the dose-response curves are similar, $\alpha$ is zero and the relative potency equals the constant $\beta$. However, if $\alpha$ is nonzero, relative potency is linear in the reciprocal of test dose, namely: $\rho_{d1}(d_1) = \hat{d}_0/d_1 = \beta + \alpha/d_1$. Later, facing data where the simple linear regression of $\hat{d}_0$ on $d_1$ seemed inadequate, DeVito et al. [18] extended their procedure to give a relative potency function that was constant up to a threshold and then linear in the reciprocal of test dose.

This approach differs in a fundamental way from Cornfield's approach. Cornfield specified two dose-response models and deduced the appropriate relative potency function. DeVito et al. specified a dose-response model for the reference chemical but not for the test chemical. Instead, by assuming a simple linear regression of $\hat{d}_0$ on $d_1$, their procedure in effect specifies a relative potency function and uses that function together with the dose-response model for the reference chemical to implicitly induce a dose-response model for the test chemical. With such a procedure, the induced dose-response model for the test chemical may not have the same functional form as the dose-response model for the reference chemical.

## *Sigmoid Dose-Response Models*

Ritz et al. [19] derived a general formula for relative potency as a function of mean response for dose-response model (1). If $f(d;\theta)$ is monotone, one can invert $\mu = f(d;\theta)$ to express dose as a function of mean response: $d = f^{-1}(\mu;\theta)$. Suppose $d_0(\mu)$ and $d_1(\mu)$ are doses of $C_0$ and $C_1$ that both produce the same mean response $\mu$. Dividing $d_0(\mu)$ by $d_1(\mu)$ expresses relative potency as a function of mean response $\mu$:

$$\rho_\mu(\mu) = f^{-1}(\mu;\theta_0)/f^{-1}(\mu;\theta_1), \tag{3}$$

where $\theta_0$ and $\theta_1$ are the parameter vectors in the dose-response models for chemicals $C_0$ and $C_1$. If $L_i$ and $U_i$ are the lower and upper response limits for $C_i$ ($i = 0, 1$), $\rho_\mu(\mu)$ is positive and finite for any $\mu$ in the intersection of the response ranges: $\max(L_0, L_1) < \mu < \min(U_0, U_1)$. Conversely, $\rho_\mu(\mu)$ is undefined for any $\mu < \min(L_0, L_1)$ or $\mu > \max(U_0, U_1)$; and if $\mu$ lies between two distinct lower (or upper) response limits, $\rho_\mu(\mu)$ is either 0 or $\infty$.

Dinse and Umbach [9] extended these ideas by expressing relative potency as functions of reference dose, of test dose, and of response quantile. Recall that similar sigmoid curves are identical up to a constant shift along the log dose axis (Fig. 1c). In fact, if we draw a horizontal arrow from the dose-response curve for $C_1$ to the dose-response curve for $C_0$, the length and direction of the arrow correspond to the magnitude and sign of the log relative potency (with left being negative). For similar dose-response curves, any horizontal arrow will have the same length and direction (Fig. 1c). For non-similar curves, each length can be distinct and the direction may change. Nevertheless, each arrow, and thus each log relative potency (or relative potency), can be indexed by mean response, reference dose, and test dose (Fig. 2). Indexing by response quantile is somewhat different, and we will return to it later.

Consider expressing relative potency as a function of dose. Substituting $f(d_0;\theta_0)$ for $\mu$ in Eq. 3 and noting that $f^{-1}(f(d_0;\theta_0);\theta_0) = d_0$, one may express relative potency as a function of reference dose $d_0$:

$$\rho_{d0}(d_0) = d_0/f^{-1}(f(d_0;\theta_0);\theta_1). \tag{4}$$

Substituting $f(d_1;\theta_1)$ instead, one may express relative potency as a function of test dose $d_1$:

$$\rho_{d1}(d_1) = f^{-1}(f(d_1;\theta_1);\theta_0)/d_1. \tag{5}$$

These relative potency functions are defined or undefined according to where the corresponding mean responses, $f(d_0;\theta_0)$ and $f(d_1;\theta_1)$, fall with respect to the bounds for $\rho_\mu(\mu)$.

Relative potency also can be indexed by response quantile (denoted by $\pi$), which is the fraction of the distance between the lower and upper response limits (i.e., mean response standardized to the unit interval). As mean response $\mu$ varies from $L$ to $U$, the corresponding quantile $\pi = (\mu - L)/(U - L)$ varies from 0 to 1. Let $ED_{100\pi}$

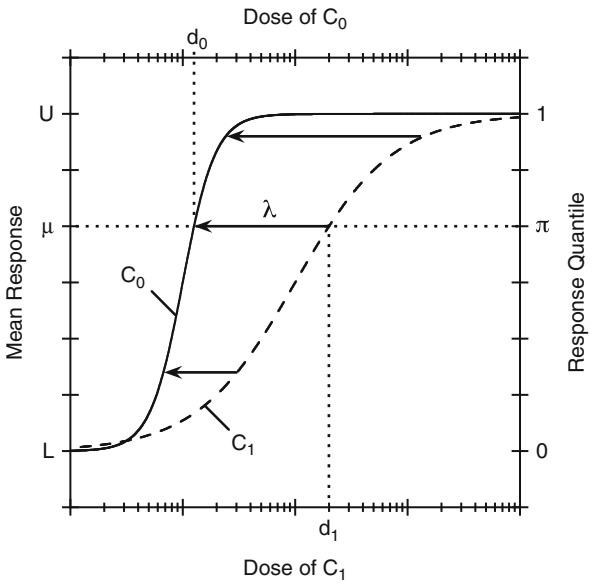


**Fig. 2** Classical definition of relative potency indexed by dose, response, and response quantile. Reference chemical $C_0$ and test chemical $C_1$ have the same lower ($L$) and upper ($U$) response limits. The length of each horizontal arrow, drawn from $C_1$ to $C_0$, varies and represents a changing log relative potency. For illustration, the arrow labeled $\lambda$ (and its corresponding relative potency) can be indexed by mean response ($\mu$), reference chemical dose ($d_0$), test chemical dose ($d_1$), or response quantile ($\pi$), as indicated by the dotted lines

be the dose producing a mean response $100\pi\%$ of the way from $L$ to $U$ (e.g., $\pi = 0.5$ gives the $ED_{50}$). If $C_0$ and $C_1$ have the same upper and same lower response limits, each value of $\pi$ corresponds to the same value of $\mu$ for both chemicals (Fig. 2). On the other hand, if the chemicals differ in one or both response limits, each value of $\pi$ will correspond to a distinct value of $\mu$ for each chemical (Fig. 3).

Consider the ratio of $ED_{100\pi}$ values for $C_0$ and $C_1$ as an alternative definition of relative potency [9]. If $C_0$ and $C_1$ have the same response limits, the log of the $ED_{100\pi}$ ratio is the horizontal distance between their dose-response curves on a log dose axis (Fig. 2). Thus, when chemicals have equal response limits, a definition based on the $ED_{100\pi}$ ratio corresponds exactly to the classical concept of relative potency. If the limits differ, however, the $ED_{100\pi}$ ratio is no longer the ratio of doses producing the same mean response. Instead, the log $ED_{100\pi}$ ratio is the horizontal component of the non-horizontal line segment connecting the dose-response curves at responses $100\pi\%$ of the way from $L_i$ to $U_i$ ($i = 0, 1$) (Fig. 3). Thus, when $C_0$ and $C_1$ have unequal limits, a definition based on the $ED_{100\pi}$ ratio embodies a modified concept of relative potency. For a given quantile $\pi$, the mean response to $C_i$ is $\mu_i = L_i + (U_i - L_i)\pi$. Dividing dose $f^{-1}(\mu_0;\theta_0)$ by dose $f^{-1}(\mu_1;\theta_1)$, Dinse and Umbach [9] obtained:

$$\rho_\pi^*(\pi) = f^{-1}\left(L_0 + (U_0 - L_0)\,\pi;\theta_0\right) / f^{-1}\left(L_1 + (U_1 - L_1)\,\pi;\theta_1\right);$$

and, under the sigmoid model in Eq. 1, they showed that $\rho_\pi^*(\pi)$ reduces to:

$$\rho_\pi^*(\pi) = g^{-1}\left(\pi;\phi_0\right) / g^{-1}\left(\pi;\phi_1\right). \tag{6}$$

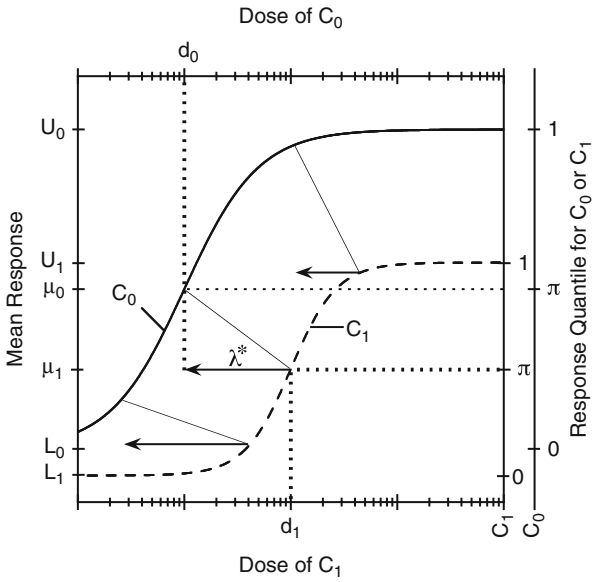


**Fig. 3** Modified definition of relative potency (based on the ratio of $ED_{100\pi}$ values) indexed by response quantile and dose. Reference chemical $C_0$ and test chemical $C_1$ have different lower ($L_0 \neq L_1$) and upper ($U_0 \neq U_1$) response limits and, hence, distinct scales for each chemical on the response quantile axis. For a selected response quantile (same $\pi$ but distinct $\mu_i$ for each chemical), an oblique line segment connects the point ($ED_{100\pi,1}$, $\mu_1$) for $C_1$ to the point ($ED_{100\pi,0}$, $\mu_0$) for $C_0$. The horizontal component (depicted by an arrow) of each oblique segment represents a modified concept of log relative potency, whose value varies with $\pi$. For illustration, the arrow labeled $\lambda^*$ (and its corresponding relative potency) can be indexed by response quantile ($\pi$), reference chemical dose ($d_0$), or test chemical dose ($d_1$), as indicated by the dotted lines

These equations express relative potency as a function of response quantile $\pi$ for any $0 < \pi < 1$. We use the modified notation $\rho^*$ to emphasize that this particular relative potency function does not, in general, embody the classical definition of relative potency.

Also, because one can index the log $ED_{100\pi}$ ratio by either the dose of the reference or test chemicals (Fig. 3), the modified definition of relative potency admits two other relative potency functions. Substituting the dose-quantile function $g(d_0;\phi_0)$ for $\pi$ in Eq. 6 and noting that $g^{-1}(g(d_0;\phi_0);\phi_0) = d_0$, one may express the modified definition of relative potency as a function of reference dose $d_0$:

$$\rho^*_{d0}(d_0) = d_0 / g^{-1}(g(d_0;\phi_0);\phi_1). \tag{7}$$

Substituting $g(d_1;\phi_1)$ instead, the modified relative potency becomes a function of test dose $d_1$:

$$\rho^*_{d1}(d_1) = g^{-1}(g(d_1;\phi_1);\phi_0)/d_1. \tag{8}$$

Equations 6, 7 and 8 express the modified relative potency as functions of response quantile $\pi$, reference dose $d_0$, and test dose $d_1$, respectively, for all $\pi \in (0,1)$, $d_0 > 0$, and $d_1 > 0$.

Consideration of the modified definition of relative potency embodied in the $\rho^*$ functions arose for two reasons. First, as mentioned in sections "Defining Relative Potency as the Ratio of $ED_{50}$s" and "Evaluating Relative Potency at Multiple $ED_{100\pi}$ Values", earlier authors have suggested using the ratio of $ED_{50}s$ or of $ED_{100\pi}$s to measure relative potency. The $\rho^*$ functions are a natural extension of those earlier approaches so examining the implications of this modified definition seemed worthwhile. Second, before fitting dose-response models to compare chemicals, toxicologists sometimes re-express measured responses as a percent of a control mean for each chemical (e.g., perhaps a zero dose is expected to give a maximal response) or rescale them to a range set by mean responses to both positive and negative control treatments (e.g., normalized percent of activation) [20]. These transformations seem designed to remove extraneous variability from the data under a belief that rescaling makes sense when comparing chemicals (a point we return to later). Thus, consideration of the $\rho^*$ functions also represented an effort to reflect common toxicologic practice, though without transforming measured responses.

Solving Eq. 5 for $f(d_1;\theta_1)$ yields $f(d_1;\theta_1) = f(d_1\rho_{d1}(d_1); \theta_0)$; that is, the dose-response function for $C_1$ can be expressed as the dose-response function for $C_0$ evaluated at dose $d_1\rho_{d1}(d_1)$. Similarly, Eq. 8 implies $g(d_1;\phi_1) = g(d_1\rho^*_{d1}(d_1); \phi_0)$. Consequently, specifying a dose-response (or dose-quantile) model and a relative potency model together is equivalent to specifying a pair of dose-response (or dose-quantile) models, a fact implicitly used by DeVito et al. [17, 18]. Recently, Dinse and Umbach [21] described conditions where modeling $\rho_{d1}(d_1)$ (or $\rho^*_{d1}(d_1)$) as a power function, $e^{\eta}d_1^{\Psi}$, guaranteed that, for a wide range of popular dose-response models, the dose-response (or, respectively, dose-quantile) models for both chemicals would have the same functional form. They also pointed out that directly modeling $\rho$ or $\rho^*$ can sometimes facilitate inferences about relative potency functions.

## *Selecting Among Various Relative Potency Functions*

The primary question is whether to use $\{\rho_\mu(\mu), \rho_{d0}(d_0), \rho_{d1}(d_1)\}$, the functions that embody the classical concept of relative potency, or $\{\rho^*_\pi(\pi), \rho^*_{d0}(d_0), \rho^*_{d1}(d_1)\}$, the functions that embody the modified concept. If the dose-response curves have identical response limits, both sets of functions are direct generalizations of the usual definition of relative potency as a ratio of equi-effective doses. Graphically these six relative potency functions convey essentially the same information because they all plot the same dose ratio as the ordinate, though each against a distinct abscissa, so the curves are differentially stretched horizontally (Fig. 4a–d).

If the response limits are not equal, however, $\{\rho^*_\pi(\pi), \rho^*_{d0}(d_0), \rho^*_{d1}(d_1)\}$, in using a modified definition of relative potency, can give a different impression than the other three relative potency functions based on the classical definition

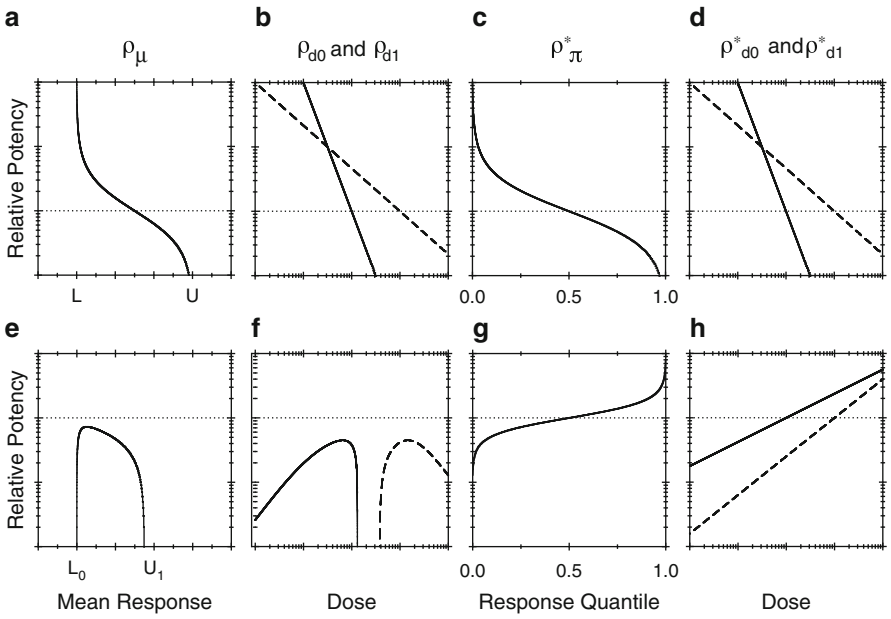


**Fig. 4** Relative potency functions corresponding to the pairs of dose-response functions in Fig. 2 (panels a–d) and in Fig. 3 (panels e–h). The relative potency functions are: $\rho_\mu(\mu)$ (panels a, e); $\rho_{d0}(d_0)$ (*solid*) and $\rho_{d1}(d_1)$ (*dashed*) (panels b, f); $\rho^*_\pi(\pi)$ (panels c, g); and $\rho^*_{d0}(d_0)$ (*solid*) and $\rho^*_{d1}(d_1)$ (*dashed*) (panels d, h). The relative potency and dose axes are logarithmic; the mean response and response quantile axes are linear. Relative potency functions fall into two equivalence classes, $\{\rho_\mu(\mu), \rho_{d0}(d_0), \rho_{d1}(d_1)\}$ and $\{\rho^*_\pi(\pi), \rho^*_{d0}(d_0), \rho^*_{d1}(d_1)\}$, corresponding to the classical definition and to a modified definition of relative potency, respectively. The dotted horizontal line in each panel represents the ratio of $ED_{50}$s (Some panels are reproduced in part from Dinse and Umbach [9])

(Fig. 4e–h). When the response limits differ, the choice between these definitions depends on whether those differences are intrinsic or extrinsic to the chemicals [9]. For example, suppose two pesticides are compared with respect to the percentage of pests killed and a subset of the population is immune to one pesticide; thus, the upper response limit would be 100% for one pesticide and less than 100% for the other. These differences are intrinsic to the chemicals and should be taken into account by using $\{\rho_\mu(\mu), \rho_{d0}(d_0), \rho_{d1}(d_1)\}$. The convenient choice is to use $\rho_\mu(\mu)$ for ranking chemicals and $\rho_{d0}(d_0)$ or $\rho_{d1}(d_1)$ for dose conversion. On the other hand, suppose each chemical's dose-response study is performed in a different laboratory. Differences in response limits would be considered extrinsic if they were idiosyncratic to the specific laboratories rather than a property of the chemicals themselves. If response-limit differences are extrinsic, $\rho^*_\pi(\pi)$ should be used for ranking chemicals because it rescales the dose-response curves to the same response range. Likewise, $\rho^*_{d0}(d_0)$ and $\rho^*_{d1}(d_1)$ would be used to calculate equivalent doses of one chemical in terms of the other on a standardized response scale. Use of

$\{\rho^*_\pi(\pi), \rho^*_{d0}(d_0), \rho^*_{d1}(d_1)\}$ is in accord with the toxicologic practice of rescaling responses as a percent of control mean response and is preferable to rescaling the data, which can introduce correlations that are not accounted for by most standard analyses.

## Example

We analyzed data from U.S. National Toxicology Program (NTP) bioassays evaluating 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) [22, 23]. We focused on cytochrome *P*450 1A1-associated 7-ethoxyresorufin-O-deethylase (EROD) activity measured in liver tissue of female Harlan Sprague–Dawley rats treated by oral gavage for 14 weeks. Both studies involved 10 rats in each of 6 dose groups (control plus 5 exposure levels). Our estimates of relative potency functions were derived from parameters estimated by fitting dose-response models using the relationships described earlier.

We analyzed log-transformed enzyme activity via least squares. We used Proc GLM in SAS (version 9.3, SAS Institute Inc., Cary, NC, USA) to fit a saturated analysis-of-variance model that estimates a mean response for each dose level of each chemical and Proc NLIN to fit nonlinear regression models. All analyses assumed a common residual variance across dose levels and chemicals. Dose-response models were Hill models based on Eq. 1 with $g(d;\phi) = d^S/(d^S + M^S)$. We compared the fit of nested models with $F$ tests [24] based on residual sums of squares and constructed simultaneous confidence bands for relative potency functions using Scheffe's method [24].

An 8-parameter model based on two separate Hill models (Table 1) showed no lack of fit (Fig. 5a, b) compared to a saturated analysis-of-variance model with 12 parameters ($F_{4,108} = 0.06$, $p = 0.99$). However, a 6-parameter model with common response limits for TCDD and PeCDF did not fit as well as the 8-parameter model ($F_{2,112} = 14.44$, $p < 0.0001$). We conclude that the chemicals have different response limits. Consider $\rho_{d1}$ as an example. If one regarded these response-limit differences as intrinsic to the chemicals, estimation of $\rho_{d1}$ as a function of PeCDF dose should use Eq. 5. The differences in response limits guarantee that $\rho_{d1}$ is non-constant. The estimated $\rho_{d1}$ is below one for most of the dose range but exceeds one at either edge of that range (Fig. 5c), suggesting that PeCDF is generally less toxic than TCDD. On the other hand, if one regarded the response-limit differences as extrinsic to the chemicals, estimation of $\rho^*_{d1}$ as a function of PeCDF dose should use Eq. 8. Relative potency modeled as a power function of PeCDF dose, $\rho^*_{d1}(d_1) = e^\eta {d_1}^\psi$, a straight line in log-log plots (Fig. 5d), fit no better than $\rho^*_{d1}(d_1) = e^\eta$ for these data ($F_{1,112} = 0.24$, $p = 0.63$) (Table 1). This conclusion is consistent with the horizontal line at 0.06 ($= e^{-2.76}$), the estimate of modified relative potency as constant, remaining within the 95% confidence band for the power-function estimate (Fig. 5d). We do not know enough about the details of

**Table 1** Parameter and standard error (SE) estimates for nested Hill dose-response models fitted to liver EROD activity [a] in rats after 14-week exposure to TCDD (reference chemical, subscripted 0) or PeCDF (test chemical, subscripted 1)[b]

| Separate 4-parameter Hill Model for Each Chemical[c] | | | | Constant relative potency (under modified definition based on $ED_{100\pi}$ ratios)[d] | |
|---|---|---|---|---|---|
| Parameterization 1 | | Parameterization 2 | | | |
| Parameter | Estimate (SE) | Parameter | Estimate (SE) | Parameter | Estimate (SE) |
| $L_0$ | 30.21 (1.96) | $L_0$ | 30.21 (1.96) | $L_0$ | 30.21 (1.96) |
| $U_0$ | 2241 (217) | $U_0$ | 2241 (217) | $U_0$ | 2151 (104) |
| $S_0$ | 0.94 (0.24) | $S_0$ | 0.94 (0.24) | $S_0$ | 1.08 (0.09) |
| $M_0$ | 4.79 (1.30) | $M_0$ | 4.79 (1.30) | $M_0$ | 4.32 (0.62) |
| $L_1$ | 48.66 (3.16) | $L_1$ | 48.66 (3.16) | $L_1$ | 48.61 (3.15) |
| $U_1$ | 2852 (389) | $U_1$ | 2852 (389) | $U_1$ | 2915 (399) |
| $S_1$ | 1.09 (0.09) | $\eta$ | −3.30 (1.05) | $\eta$ | −2.76 (0.27) |
| $M_1$ | 65.06 (18.3) | $\psi$ | 0.16 (0.31) | | |
| MSE (df)[e] | 0.0422 (112) | | | 0.0420 (113) | |

[a]Activity measured as nmol of resorufin formed per min per mg of microsomal protein

[b]Doses measured as ng per kg of body weight per day

[c]The parameterizations are related by: $\psi = S_1/S_0 - 1$ and $\eta = log(M_0) - (S_1/S_0)log(M_1)$. The first parameterization is used to estimate $\rho_{d1}(d_1)$ via Eq. 5. The second parameterization is used to estimate $\rho^*_{d1}(d_1) = e^{\eta} d_1{}^{\psi}$

[d]Relative potency (modified definition) is constant: $\rho^*_{d1}(d_1) = e^{\eta}$

[e]Mean Squared Error, an estimate of the residual variance, and associated degrees of freedom

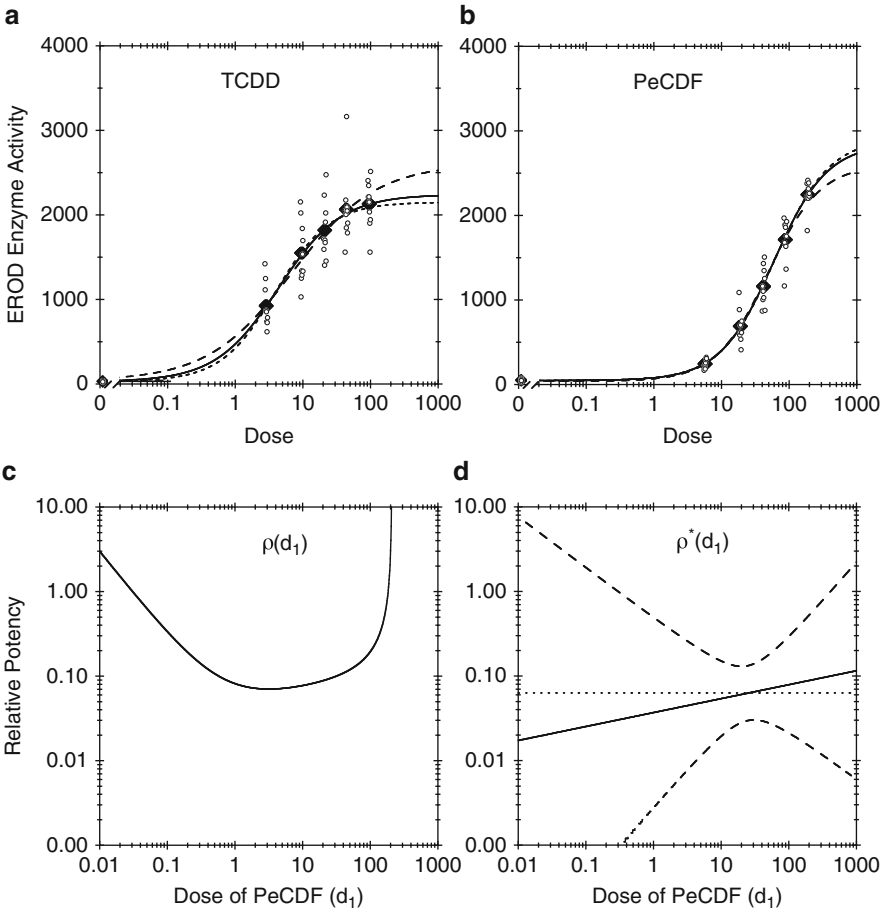


**Fig. 5** Dose-response and relative potency for TCDD and PeCDF for liver EROD activity (pmol of resorufin formed per min per mg of microsomal protein) in rats after 14-week exposure via oral gavage. Dose units are ng per kg of body weight per day. Panels (**a**) TCDD and (**b**) PeCDF show observed activity for each rat (○), dose-specific means (♦), and estimated dose-response curves (solid, 8-parameter model with a separate Hill function for each chemical; dashed, 6-parameter model honoring a constraint that both chemicals have same response limits; dotted, 7-parameter model honoring a constraint that $\rho_{d1}^{*}(d_1)$ is constant). Panel (**c**) shows an estimate of $\rho_{d1}(d_1)$. Panel (**d**) shows estimates of $\rho_{d1}^{*}(d_1)$ (solid, as a power-function; dashed, its 95% simultaneous confidence band; dotted, as a constant)

the experiments and the biology to decide whether the response-limit differences should be regarded as intrinsic or extrinsic to these chemicals. Regardless of that judgment, however, these data support a conclusion that PeCDF is less potent than TCDD.

## Summary

The idea that relative potency should be constant is rooted historically in analytical dilution assays. It simplifies chemical ranking and dose conversion. If relative potency is a constant equal to $\rho$, the dose of chemical $C_0$ that is equivalent to dose $d_1$ of chemical $C_1$ is $d_0 = d_1\rho$, and the dose of $C_1$ that is equivalent to dose $d_0$ of $C_0$ is $d_1 = d_0/\rho$. Chemical ranking is even easier: order each chemical by its value of $\rho$.

Toxicologists, however, have long been faced with data from comparative assays that indicate that relative potency is not generally constant. Over the years, various investigators have suggested ways to cope with non-constant relative potency. Extending the concept that relative potency is the ratio of equi-effective doses, Cornfield [16] showed that linear dose-response models in log dose induce relative potency functions that are log-linear in log dose or response. In fact, a wide variety of monotone dose-response models can be inverted to express relative potency as a function of reference dose, test dose, or mean response. Analogously, using a modified concept of relative potency as the ratio of $ED_{100\pi}$s, one can express (modified) relative potency as a function of reference dose, test dose, or response quantile. If the chemicals have the same response limits, the classical and modified definitions of relative potency coincide. Relative potency functions allow chemicals to be ranked with respect to toxicity, though that ranking may change for different dose or response levels. For dose conversion, the dose of $C_0$ that is equivalent to dose $d_1$ of $C_1$ is $d_0 = d_1\rho_{d1}(d_1)$ and the dose of $C_1$ that is equivalent to dose $d_0$ of $C_0$ is $d_1 = d_0/\rho_{d0}(d_0)$. The choice between $\{\rho^*_\pi(\pi), \rho^*_{d0}(d_0), \rho^*_{d1}(d_1)\}$, based on the modified concept of relative potency, and $\{\rho_\mu(\mu), \rho_{d0}(d_0), \rho_{d1}(d_1)\}$, based on the classical definition, depends on whether response limits differ for extrinsic or intrinsic reasons. Relative potency functions appear to be a promising avenue for characterizing non-constant relative potency.

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