Parameterizing Dose-Response Models to Estimate Relative Potency Functions Directly

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Many comparative analyses of toxicity assume that the potency of a test chemical relative to a reference chemical is constant, but employing such a restrictive assumption uncritically may generate misleading conclusions. Recent efforts to characterize non-constant relative potency rely on relative potency functions and estimate them secondarily after fitting dose-response models for the test and reference chemicals. We study an alternative approach of specifying a relative potency model *a priori* and estimating it directly using the dose-response data from both chemicals. We consider a power function in dose as a relative potency model and find that it keeps the two chemicals' dose-response functions within the same family of models for families typically used in toxicology. When differences in the response limits for the test and reference chemicals are attributable to the chemicals themselves, the older two-stage approach is the more convenient. When differences in response limits are attributable to other features of the experimental protocol or when response limits do not differ, the direct approach is straightforward to apply with nonlinear regression methods and simplifies calculation of simultaneous confidence bands. We illustrate the proposed approach using Hill models with dose-response data from U.S. National Toxicology Program bioassays. Though not universally applicable, this method of estimating relative potency functions directly can be profitably applied to a broad family of dose-response models commonly used in toxicology.

Key Words: dose response; Hill model; PeCDF; relative potency; TCDD.

Toxicologists often compare the potency of one chemical to another and use relative potency values for ranking chemicals or for converting doses of a test chemical into their equivalents for a reference chemical. Relative potency is also used when evaluating mixtures of chemicals for dose additivity or when calculating toxic equivalency factors.

Usually, relative potency is taken as constant, an assumption that arises naturally in the context of dilution assays (Finney, 1965). When two chemicals exhibit constant relative potency, their dose-response curves plotted on a log-dose axis are identical up to a constant horizontal shift; relative potency depends on

the magnitude and direction of that shift. Comparative assays often, however, involve dose-response curves whose horizontal separation is non-constant (Cornfield, 1964; De Lean *et al.*, 1978; DeVito *et al.*, 2000; Guardabasso *et al.*, 1988). In those situations, methods that accommodate non-constant relative potency are desirable to avoid misleading conclusions.

Recent work focuses on relative potency functions as general descriptors of non-constant relative potency (Dinse and Umbach, 2011; Ritz *et al.*, 2006). The fundamental idea is that possibly varying horizontal distances between two dose-response curves may be indexed by any one of several quantities: dose of either the test or reference chemical, mean response, or response quantile. Thus, a relative potency function expresses local relative potency across the dose or response range as a function of one of these quantities, thereby providing a global description of the relative potency relationship.

In earlier work, we postulated a dose-response model for each chemical and expressed relative potency functions in terms of the parameters from those dose-response models (Dinse and Umbach, 2011). For example, if the dose-response relationship for each chemical obeyed a four-parameter model, the relative potency function would be expressed using all eight parameters. Of course, fewer parameters suffice if some parameters are constrained as equal when fitting the models. This original approach may be characterized as a two-stage process that initially estimates parameters for a pair of dose-response functions and then plugs those estimates into an appropriate expression to estimate the relative potency function. The relative potency function contains no fundamentally new information beyond that contained in the dose-response functions—it simply describes a relationship between them.

Here, we propose an alternative approach for estimating relative potency functions. This alternative specifies a relative potency function *a priori* and estimates it directly using standard nonlinear regression methods. This approach requires specification of a dose-response model for the reference chemical and a relative potency model (these two determine the dose-response model for the test chemical). When the relative potency function is the

principal object of study, specifying a model for it explicitly has intuitive appeal. This proposed approach is motivated by a desire to simplify statistical inference on relative potency functions; estimation and testing should be more straightforward when a relative potency model is specified directly.

Selecting a model for relative potency requires care, however. Comparative assays generally employ monotone dose-response models for the test and reference chemicals that are within the same family (i.e., have the same functional form but possibly different parameter values). Matching an arbitrary relative potency model to a given dose-response model for the reference chemical might induce a dose-response function for the test chemical that is in a different family of models or even one that is non-monotone. Consequently, we focus on relative potency models that we call “compatible” in that they keep the dose-response models for both reference and test chemicals within the same family.

Our purpose is to develop this alternative approach and explore its strengths and limitations. We consider monotone dose-response models parameterized by lower and upper response limits, plus a vector of additional parameters. We consider two situations: one where any observed differences in response limits are attributable to the chemicals themselves (*intrinsic* differences) and another where such differences arise from other aspects of the experimental procedure (*extrinsic* differences). We find that, when differences in response limits are intrinsic, specifying a compatible relative potency function is difficult so the two-stage approach is more convenient. Alternatively, if the chemicals have the same response limits or if any observed differences are seen as extrinsic, compatible relative potency functions are easy to specify for commonly used dose-response models. Thus, the explicit specification of relative potency functions is widely, though not universally, applicable. We use data from U.S. National Toxicology Program (NTP) bioassays to illustrate the direct estimation of relative potency functions.

MATERIALS AND METHODS

Dose-response models. Let $f(d; \theta)$ denote the mean response elicited by non-negative dose d of a chemical of interest. Here, f , the dose-response model, is a monotone function of d that depends on a vector θ of unknown parameters. We focus on dose-response curves for which the mean response increases from a lower limit or background level L at a dose of zero ($d = 0$) to an upper limit U at an infinite dose ($d = \infty$). (If, instead, response decreases with dose, then U is associated with $d = 0$ and L with $d = \infty$.) We express such dose-response curves using the general formula:

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

where g is a monotone function that ranges from 0 to 1 as d ranges from 0 to ∞ and depends on a vector ϕ of unknown parameters. Here, $\theta = (L, U, \phi)$. Various functions that meet the requirements for g are commonly used in toxicology (Table 1). For our illustrations, we will use the Hill (1910) model, a sigmoid function often used for dose-response relationships, where

$$g(d; \phi) = d^S / (d^S + M^S) = \{1 + \exp[S \log(M) - S \log(d)]\}^{-1}. \quad (2)$$

TABLE 1
Dose-Quantile Functions for Several Dose-Response Models Whose Compatible Relative Potency Model Is a Power Function in Dose^a

Dose-response model	Dose-quantile function	
	$g(d; \phi)^b$	$h(X)^c$
Logistic/Hill	$[1 + \exp(\alpha + \beta \log(d))]^{-1}$	$[1 + \exp(X)]^{-1}$
Weibull	$1 - \exp[-\exp(\alpha + \beta \log(d))]$	$1 - \exp[-\exp(X)]$
Probit ^d	$\Phi(\alpha + \beta \log(d))$	$\Phi(X)$
Generalized logistic/Hill	$[1 + \exp(\alpha + \beta \log(d))]^{-\gamma}$	$[1 + \exp(X)]^{-\gamma}$
Generalized Weibull	$\{1 - \exp[-\exp(\alpha + \beta \log(d))]\}^{-\gamma}$	$\{1 - \exp[-\exp(X)]\}^{-\gamma}$

^aA power function in dose has form $\rho^\circ(d) = e^{\eta} d^{\psi}$ or, equivalently, $\log[\rho^\circ(d)] = \eta + \psi \log(d)$.

^bThe vector ϕ contains parameters α and β and, optionally, additional parameters γ .

^cThe dose-quantile function $g(d; \phi)$ is obtained by substituting $X = \alpha + \beta \log(d)$ into $h(X)$.

^d $\Phi(X) = (2\pi)^{-1/2} \int_{-\infty}^X \exp(-t^2/2) dt$ is the standard normal distribution function.

Here, $\phi = (S, M)$, where S regulates the shape of the dose-response curve and M is the median effective dose (or ED_{50}). For convenience, we use the notation “log” for the natural logarithm.

Relative potency. Let subscripts 0 and 1 index the reference and test chemicals, respectively. In dilution assays where relative potency (denoted ρ) is constant, the dose-response functions (denoted f_0 and f_1 , for the reference and test chemicals, respectively) must have the same functional form (say, f) though they each have distinct parameter vectors θ_0 and θ_1 , respectively. When relative potency is constant, then $f(d_1; \theta_1) = f(\rho d_1; \theta_0)$ for any dose $d_1 \geq 0$ (Finney, 1965). This equation states that, at any specified dose d_1 of the test chemical, a dose d_0 of the reference chemical equal to a constant ρ times d_1 yields the same mean response as a dose d_1 of the test chemical. Consequently, $\rho = d_0/d_1$, where d_0 and d_1 are any two doses of the respective chemicals that elicit the same mean response. Equipotency corresponds to $\rho = 1$.

Recent efforts to accommodate non-constant relative potency are based on relative potency functions (Dinse and Umbach, 2011; Ritz *et al.*, 2006). When plotted with dose on a logarithmic axis, the horizontal distance between two dose-response curves at a given response level μ corresponds to the magnitude of the log-relative potency at that value of μ , i.e., $\log(\rho) = \log(d_0) - \log(d_1)$. When that horizontal distance changes along the curves, it (or, equivalently, the relative potency itself) can be indexed by the mean response, the dose of the reference chemical, or the dose of the test chemical. The resulting three relative potency functions—denoted $\rho_\mu(\mu)$, $\rho_{d_0}(d_0)$, and $\rho_{d_1}(d_1)$, respectively, by Dinse and Umbach (2011)—describe the same fundamental relative potency information but through different variables.

Let response quantile π be defined as response level μ expressed as a fractional distance from the lower response limit L to the upper response limit U , i.e., $\pi = (\mu - L)/(U - L)$. As μ ranges from L to U , π ranges from 0 to 1. We call the function $g(d; \phi)$ in Equation 1 a *dose-quantile* model, a dose-response model that gives the response quantile as a function of dose. Let $ED_{100\pi}$ denote the dose that produces a response 100 $\pi\%$ of the distance from L to U . When the two dose-response functions have the same upper and the same lower response limits, the relative potency at response level μ with corresponding response quantile π is simply the ratio of the $ED_{100\pi}$ values, reference over test. Consequently, for chemicals with identical response limits, relative potency also can be indexed by π , leading to another relative potency function that we denote $\rho_\pi^*(\pi)$. When chemicals differ in their response limits, one can still index the ratio of $ED_{100\pi}$ values by π and so construct a function $\rho_\pi^*(\pi)$; however, with unequal response limits, the ratio of $ED_{100\pi}$ values no longer corresponds to the classical definition of relative potency but represents a modified definition

(Dinse and Umbach, 2011). The ratio of $ED_{100\pi}$ values can alternatively be indexed by the corresponding values of d_0 or of d_1 , leading to two additional functions $\rho_{d_0}^*(d_0)$ and $\rho_{d_1}^*(d_1)$, respectively (Dinse and Umbach, unpublished manuscript). We employ the notation ρ^* for these functions to emphasize that, in general, they embody a modified definition of relative potency, one that coincides with the classical definition only when the two dose-response functions share both upper and lower response limits.

Though a modified definition of relative potency may initially seem undesirable, the utility of the ρ^* functions emerges when differences in response limits are believed to be extrinsic to the chemicals themselves, arising idiosyncratically from experimental differences such as batch-to-batch or laboratory-to-laboratory effects. In such circumstances, investigators often rescale data from each chemical to the same response limits before assessing relative potency. The ρ^* functions accommodate this rescaling in their definition, without manipulating the data itself. That is, when response limits differ between chemicals, dose-response curves can be fit to data without rescaling, but relative potency functions appropriate when investigators believe response limits are equal may be deduced nonetheless.

The relative potency function $\rho_{d_1}(d_1)$, which we abbreviate as $\rho(d_1)$, expresses changing values of relative potency as a function of dose d_1 of the test chemical. Following Equation A.4 in Dinse and Umbach (2011), we have

$$\rho(d_1) = f_0^{-1}(f_1(d_1; \theta_1); \theta_0)/d_1, \quad (3)$$

where, for convenience, the notation $\rho(d_1)$ suppresses the dependence on parameter vectors θ_0 and θ_1 . Here, f^{-1} denotes the inverse function of f (not the reciprocal). The monotone dose-response function f expresses mean response level μ as a function of dose d ; the inverse function inverts the relationship and expresses dose d as a monotone function of mean response level μ . Equation 3 holds for all positive values of d_1 such that both $f_1(d_1; \theta_1)$ and $f_0(d_1 \rho(d_1); \theta_0)$ are between $\max(L_0, L_1)$ and $\min(U_0, U_1)$, that is, the range of response common to both chemicals.

Similarly, the modified relative potency function $\rho_{d_1}^*(d_1)$, which we abbreviate as $\rho^*(d_1)$, is given by Equation 10 in Dinse and Umbach (unpublished manuscript) as

$$\rho^*(d_1) = g_0^{-1}(g_1(d_1; \phi_1); \phi_0)/d_1, \quad (4)$$

where, for convenience, the notation $\rho^*(d_1)$ suppresses the dependence on parameter vectors ϕ_0 and ϕ_1 . Equation 4 holds for all positive values of d_1 .

For the postulated dose-response models f_0 and f_1 , after having estimated their parameters $\theta_0 = (L_0, U_0, \phi_0)$ and $\theta_1 = (L_1, U_1, \phi_1)$ from experimental data, one can estimate the corresponding relative potency function by plugging those estimated values of θ_0 and θ_1 into the right side of Equation 3 or Equation 4. This process is the essence of what was described in the introduction as the two-stage approach to estimating a relative potency function.

Experimental data. The U.S. NTP recently evaluated toxicity and carcinogenicity endpoints for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the dioxin-like compound 2,3,4,7,8-pentachlorodibenzofuran (PeCDF; National Toxicology Program, 2006a,b). We analyzed a subset of the data from these 2-year bioassays (data provided in Supplementary data). Specifically, we focused on the activity of cytochrome *P*450 1A1-associated 7-ethoxyresorufin-O-deethylase (EROD) and cytochrome *P*450 1A2-associated acetanilide-4-hydroxylase (A4H) as measured in liver tissue of female Harlan Sprague Dawley rats treated by oral gavage for 53 weeks. EROD activity was measured as pmol of resorufin per min per mg microsomal protein; A4H activity, as nmol of 4-hydroxyacetanilide formed per min per mg of microsomal protein; and dose, as ng per kg of body weight per day. Each study involved eight rats in each of six dose groups (control plus five exposure levels), with PeCDF doses being twice as large as TCDD doses.

Statistical analysis. We analyzed log-transformed enzyme activity via least squares. To fit a saturated analysis-of-variance model that estimates a mean response for each dose level of each chemical, we used PROC GLM in SAS (version 9.2, SAS Institute Inc., Cary, NC). To fit nonlinear regression models, we used SAS PROC NLIN. All analyses assumed a common residual

variance across dose levels and chemicals, and they employed a Hill model with the dose-quantile function $g(d; \phi)$ of Equation 2.

We compared fit between two nested models with an F test (Seber and Wild, 1989) based on residual sums of squares. We constructed simultaneous confidence bands for the relative potency functions using the Scheffe method (Seber and Wild, 1989). Annotated SAS code that can be used to perform all the analyses described here as well as additional detail about tests of fit and confidence band calculation is available in Supplementary data.

RESULTS

Relative Potency Model

Rather than estimating the relative potency function in two stages using parameters estimated from previously fitted dose-response models, we propose an alternative approach of modeling the relative potency function explicitly and estimating it directly as an integral part of fitting the dose-response models. Thus, instead of specifying dose-response models for both the reference and test chemicals, we specify a dose-response model for the reference chemical and a model for relative potency as a function of dose of the test chemical. Together these induce a dose-response relationship for the test chemical. In practice, our proposal amounts to a reparameterization of the test chemical's dose-response model. Often one has more and/or better information about the reference chemical than the test chemical; so, modeling the dose-response relationship for the reference chemical is reasonable. If the goal is to estimate the potency of a test chemical relative to a reference chemical, then modeling $\rho(d_1)$ directly rather than obtaining an estimate indirectly from two dose-response models is often convenient.

Parallel to Finney's (1965) demonstration that $f(d; \theta_1) = f(\rho d; \theta_0)$ for dose-response curves with constant relative potency, one can derive a corresponding expression for curves where relative potency is not constant. Multiplying both sides of Equation 3 by d_1 and then applying function f_0 to both sides of that result yields

$$f_1(d_1; \theta_1) = f_0(d_1 \rho(d_1); \theta_0) \text{ for any } d_1 \text{ for which } \rho(d_1) \text{ is defined.} \quad (5)$$

That is, the dose-response function for the test chemical at dose d_1 can be expressed as the dose-response function for the reference chemical evaluated at a dose given by $d_1 \rho(d_1)$. In this way, the dose-response model for the test chemical is induced by a dose-response model for the reference chemical and a specified relative potency function. Similar operations applied to Equation 4 yield

$$g_1(d_1; \phi_1) = g_0(d_1 \rho^*(d_1); \phi_0) \text{ for any } d_1 \geq 0. \quad (6)$$

Thus, the dose-quantile function for the test chemical at dose d_1 can be expressed as the dose-quantile function for the reference chemical evaluated at a dose given by $d_1 \rho^*(d_1)$. The convenience of employing either Equation 5 or Equation 6 arises when a simple functional form is appropriate for modeling $\rho(d_1)$ or $\rho^*(d_1)$.

By either the classical or modified definition, relative potency is a ratio of doses; thus, the function $\rho(d_1)$ or $\rho^*(d_1)$ must be non-negative. In addition, relative potency functions are further restricted because any function employed must allow f_1 and g_1 to be monotone. Finally, in most analyses of relative potency, a common functional form is fitted to both the reference and test chemicals. We describe relative potency functions that honor this requirement as *compatible* with the given dose-response model. To be compatible with f_0 , any relative potency function applied in Equation 5 must allow f_1 to maintain the same functional form as f_0 , with an analogous requirement for g_0 and g_1 , in Equation 6.

In general, specification of a relative potency model that meets all these requirements is difficult. As illustrated in Figure 4 of Dinse and Umbach (2011), when each dose-response model is a Hill model, $\rho(d_1)$ can take on various shapes and need not even be monotone. In their most general forms, such relative potency functions are too algebraically complex for the direct modeling considered here. If, however, both Hill models have the same response limits, the compatible $\rho(d_1)$ has a power-function form in d_1 —equivalently, $\log(\rho(d_1))$ has a linear form in $\log(d_1)$ (Dinse and Umbach, 2011). In addition, the same power-function form for $\rho^*(d_1)$ provides the most complex form possible for $\rho^*(d_1)$ to be compatible with a Hill dose-response model. Consequently, we propose the following power function for either $\rho(d_1)$ or $\rho^*(d_1)$:

$$\rho^\circ(d_1) = e^\eta d_1^\psi = \exp\{\eta + \psi \log(d_1)\}. \quad (7)$$

Here, η and ψ are unknown parameters to be estimated and the notation $\rho^\circ(d_1)$ omits explicit mention of parameters and indicates the form applies to $\rho(d_1)$ or $\rho^*(d_1)$. If $\psi = 0$, Equation 7 reduces to the constant relative potency model: $\rho^\circ(d_1) = e^\eta = \rho$ for any test dose d_1 ; otherwise, it allows relative potency to increase ($\psi > 0$) or decrease ($\psi < 0$) monotonically with d_1 .

For example, using Equation 7 to model $\rho(d_1)$ and taking the dose-response model for the reference chemical as $f_0(d_0; \theta_0)$, the induced dose-response model for the test chemical, according to Equation 5, is $f_1(d_1; \theta_1) = f_0(e^\eta d_1^\psi; \theta_0)$. In this case, the parameter vector θ_1 consists of (θ_0, η, ψ) —indicating that any parameters in θ_0 must also appear in θ_1 . Assume further that $f_0(d_0; \theta_0)$ is a Hill model specified using Equation 1 with $g_0(d_0; \phi_0)$ from Equation 2 and $\theta_0 = (L_0, U_0, \phi_0)$ and $\phi_0 = (M_0, S_0)$. Employing this specification yields

$$f_1(d_1; \theta_1) = L_0 + (U_0 - L_0) \{1 + \exp[S_0 \log(M_0) - S_0 \log(e^\eta d_1^\psi)]\}^{-1}.$$

Rearranging the terms within the braces gives

$$f_1(d_1; \theta_1) = L_0 + (U_0 - L_0) \{1 + \exp[S_0(\psi+1) \log(M_0 e^{-\eta})^{1/(\psi+1)} - S_0(\psi+1) \log(d_1)]\}^{-1}.$$

This dose-response model for the test chemical is also a Hill model with the same response limits as the reference chemical, namely, L_0 and U_0 , but with distinct ED_{50} and shape parameters, $M_1 = (M_0 e^{-\eta})^{1/(\psi+1)}$ and $S_1 = S_0(\psi+1)$, respectively. In modeling $\rho(d_1)$, the response limits are forced to be the same for

the reference and test chemicals. Thus, explicit fitting of $\rho(d_1)$ should only be done when the data are consistent with common response limits for both chemicals.

Following a similar development, but using Equation 7 to model $\rho^*(d_1)$ and taking the dose-quantile model for the reference chemical as $g_0(d_0; \phi_0)$, the induced dose-quantile model for the test chemical, according to Equation 6, is $g_1(d_1; \phi_1) = g_0(e^\eta d_1^\psi; \phi_0)$. The parameter vector ϕ_1 consists of (ϕ_0, η, ψ) —indicating that any parameters in ϕ_0 must also appear in ϕ_1 . Assume further that the reference chemical's dose-response model is a Hill model with $g_0(d_0; \phi_0)$ from Equation 2 and $\phi_0 = (M_0, S_0)$. This specification yields

$$g_1(d_1; \phi_1) = \{1 + \exp[S_0(\psi+1) \log(M_0 e^{-\eta})^{1/(\psi+1)} - S_0(\psi+1) \log(d_1)]\}^{-1}.$$

Thus, the induced dose-quantile model for the test chemical corresponds to that of a Hill model but with $\phi_1 = (M_1, S_1)$, where M_1 and S_1 are defined in the preceding paragraph. Notice that, by modeling $\rho^*(d_1)$, one induces a dose-quantile model for the test chemical, but does not induce the full dose-response model, that is, the response limits remain unspecified. Thus, direct fitting of $\rho^*(d_1)$ is possible when the data indicate different response limits for the two chemicals, in accord with the definition of $\rho^*(d_1)$. In fact, fitting $\rho^*(d_1)$ in this way is a reparameterization equivalent to fitting different four-parameter Hill models for each chemical.

The relative potency function of Equation 7 is compatible with an array of dose-response functions; it is not restricted to the Hill model, our focus to this point. Each of the dose-quantile models listed in Table 1 can be seen to depend on $\log(d)$ through the linear form $\alpha + \beta \log(d)$, where α and β are parameters to be estimated (of course, the dose-quantile model g may involve additional parameters, say γ). Accordingly, we may write

$$g(d; \phi) = h(\alpha + \beta \log(d); \gamma) \quad (8)$$

for some monotone function h and for parameter vector $\phi = \{\alpha, \beta, \gamma\}$. If g_0 has that form, then using Equation 6 with the power function of Equation 7 for $\rho^*(d_1)$ yields

$$g_1(d_1; \phi_1) = g_0(d_1 \rho^*(d_1); \phi_0) = h(\alpha + \beta \log(e^\eta d_1^\psi); \gamma) = h([\alpha + \beta \eta] + [\beta(\psi+1)] \log(d_1); \gamma).$$

Thus, g_1 has the same functional form as g_0 , namely, the form of Equation 8 but with different parameter values: $\phi_1 = \{[\alpha + \beta \eta], [\beta(\psi+1)], \gamma\}$ instead of $\phi_0 = \{\alpha, \beta, \gamma\}$. Consequently, $\rho^*(d_1)$ of Equation 7 is compatible with any dose-quantile function that has the form of Equation 8. Notice that γ has the same value for both g_0 and g_1 ; so that meaningful application of Equation 6 to dose-quantile functions like the generalized logit (Table 1) requires that both chemicals have a common value of any such additional parameters. Of course, a parallel argument starting from Equation 5 shows that $\rho(d_1)$ from Equation 7 is compatible with dose-response functions based on dose-quantile functions whose form matches Equation 8. In this case, however, the upper and lower response limits as well as any additional

parameter γ would necessarily have the same values for both the reference and test chemicals.

General Strategy for Data Analysis

To fix a context, assume that a comparative toxicity study involving two chemicals has probed several dose levels of each chemical using multiple replicate units per dose. Assume further that a suitable transformation has provided a response measure whose variance is constant across doses and chemicals. All the models that we describe are fitted to the combined data from both chemicals in a single least-squares minimization. We describe the strategy in terms of Hill models but, of course, many other dose-response functions would serve.

As a baseline for assessing goodness-of-fit for subsequent models, first fit a saturated analysis-of-variance model that estimates a mean response for each dose-chemical combination. Second, fit a model with 8 parameters (L_0 , U_0 , S_0 , M_0 , L_1 , U_1 , S_1 , and M_1), based on two 4-parameter Hill functions, and confirm that this second model does not significantly degrade fit compared with the saturated model. (If the fit has degraded, examine other dose-response functions in search of an adequate one.) Third, with a dose-response function that fits adequately, examine whether both chemicals have the same response limits. That is, fit a reduced model using six parameters (L , U , S_0 , M_0 , S_1 , and M_1) that enforces common response limits for both chemicals. If this model fits as well as the eight-parameter model, conditions are met for directly fitting a power function for relative potency, i.e., for $\rho(d_1)$; the resulting dose-response model is a reparameterization of the previous six-parameter model, now with parameters (L , U , S_0 , M_0 , η , and ψ). One can further reduce this model to five parameters (L , U , S_0 , M_0 , and η) to examine whether relative potency is constant.

If the enforcement of common response limits degrades fit compared with the eight-parameter model, one faces a choice. Under a belief that any differences in response limits are intrinsic to the chemicals themselves (i.e., those differences arise from differential action of the chemicals), the relative potency function $\rho(d_1)$ is meaningful and can be estimated by plugging the eight estimated parameters into formulas in Dinse and Umbach (2011). On the other hand, under a belief that differences in response limits are extrinsic to the chemicals and arise from idiosyncrasies of the experiments, the modified relative potency function $\rho^*(d_1)$ is meaningful and can be estimated directly via a reparameterization of the eight-parameter model using (L_0 , U_0 , S_0 , M_0 , L_1 , U_1 , η , and ψ). One can reduce this model to seven parameters (L_0 , U_0 , S_0 , M_0 , L_1 , U_1 , and η) to examine whether modified relative potency is constant.

Illustration Using NTP Data

Following the strategy in the preceding subsection, we illustrate the direct estimation of $\rho(d_1)$ or $\rho^*(d_1)$ using data from NTP studies. Consider first the potency of PeCDF relative to TCDD with respect to liver EROD activity after 53 weeks of

TABLE 2
Parameter and Standard Error (SE) Estimates for Two Parameterizations of a Pair of Hill Dose-Response Models Fitted to Liver EROD Activity^a in Rats after 53-Week Exposure to TCDD (Reference Chemical, Subscripted 0) or PeCDF (Test Chemical, Subscripted 1)^b

Parameterization 1 ^c		Parameterization 2 ^c	
Parameter	Estimate (SE)	Parameter	Estimate (SE)
L_0	29.92 (2.01)	L_0	29.92 (2.01)
U_0	1863 (131.5)	U_0	1863 (131.5)
S_0	1.25 (0.22)	S_0	1.25 (0.22)
M_0	6.05 (1.11)	M_0	6.05 (1.11)
L_1	77.17 (5.19)	L_1	77.17 (5.19)
U_1	6960 (2623)	U_1	6960 (2623)
S_1	0.81 (0.09)	η	-1.65 (0.50)
M_1	207.6 (170.4)	ψ	-0.35 (0.13)
MSE (df) ^d	0.0362 (88)		

^aActivity measured as pmol of resorufin formed per min per mg of microsomal protein.

^bDoses measured as ng per kg of body weight per day.

^cThe two parameterizations of the eight-parameter model are related by $\psi = S_1/S_0 - 1$ and $\eta = \log(M_0) - (S_1/S_0) \log(M_1)$. The second parameterization estimates the relative potency function $\rho^*(d_1)$ directly through parameters η and ψ .

^dMean Squared Error, an estimate of residual variance, and associated degrees of freedom.

exposure via oral gavage. Separate Hill models, equivalent to a single 8-parameter model, exhibited no lack-of-fit to the observed data compared with a saturated analysis-of-variance model with 12 parameters ($F_{4,84} = 0.26$, $p = 0.90$; Figs. 1A and 1B). Not surprisingly, given that TCDD and PeCDF had disparate estimated response limits (Table 2), a six-parameter model with common response limits for both chemicals showed statistically significant lack-of-fit compared with the eight-parameter model ($F_{2,88} = 60.90$, $p < 0.0001$). If we regarded the differences in response limits as intrinsic to the chemicals, the disparate response limits imply that estimation of $\rho(d_1)$ must be done in two stages through Equation 3 specialized to two Hill models. This estimate $\hat{\rho}(d_1)$ was below 1 for doses between 0.70 and 50.85 ng PeCDF per kg body weight per day; otherwise $\hat{\rho}(d_1)$ exceeded 1, growing increasingly large as dose approached 0 or 56.93 ng PeCDF per kg body weight per day, the bounds where $\hat{\rho}(d_1)$ is defined (Fig. 1C). On the other hand, if we regarded the differences in response limits as extrinsic to the chemicals—as a nuisance that should be ignored—attention would focus on $\rho^*(d_1)$ instead of on $\rho(d_1)$. Assuming that $\rho^*(d_1)$ had the power-function form of Equation 7, we estimated it directly through a reparameterization of the eight-parameter model (Table 2). This estimated relative potency function was below 1 for doses greater than ~0.01 ng PeCDF per kg of body weight per day and fell farther below 1 at higher doses (Fig. 1D). Finally, eliminating ψ from the eight-parameter model resulted in a statistically significant degradation of fit ($F_{1,88} = 4.13$, $p = 0.045$), indicating that $\rho^*(d_1)$ is not constant for these data. This conclusion

FIG. 1. Dose-response and relative potency for TCDD and PeCDF derived from liver EROD activity in rats after 53-week exposure via oral gavage. Enzyme activity was measured as pmol of resorufin formed per min per mg of microsomal protein; dose was measured in ng per kg of body weight per day. Panels (a) TCDD and (b) PeCDF show observed activity for each animal ($\circ$), dose-specific means ($\bullet$), and estimated dose-response curves (solid, 8-parameter model with a separate Hill function for each chemical; dashed, 6-parameter model honoring constraint that both chemicals have same response limits; dotted, 7-parameter model honoring constraint of constant $\rho^*(d_1)$). Panel (c) shows two-stage estimate of $\rho(d_1)$ based on estimates from 8-parameter model. Panel (d) shows direct estimates of $\rho^*(d_1)$ (solid, enforcing power-function model for relative potency under the belief that differences in response limits are extrinsic to the chemicals themselves; dashed, its 95% simultaneous confidence band; dotted, enforcing constant relative potency under the same belief).

could also be reached by noting that the horizontal line at 0.08 ($= e^{-2.54}$), the estimate of constant $\rho^*(d_1)$, crosses the 95% confidence band for the power-function estimate of $\rho^*(d_1)$ at a PeCDF dose of $d_1 = 0.02$ (Fig. 1D). We conclude that PeCDF is generally less potent than TCDD with respect to liver EROD activity and that relative potency declines as dose increases.

Consider next the potency of PeCDF relative to TCDD with respect to liver A4H activity after 53 weeks of exposure via oral gavage. Separate Hill models (an 8-parameter model) exhibited no lack-of-fit to the observed data compared with a saturated analysis-of-variance model with 12 parameters ($F_{4,84} = 1.26$, $p = 0.29$; Figs. 2A and 2B). The two-stage estimate $\hat{\rho}(d_1)$ based on the eight-parameter model was below 1 for

doses between 0.68 and 194.24 ng PeCDF per kg body weight per day; otherwise $\hat{\rho}(d_1)$ exceeded 1, growing increasingly large as dose approached 0 or 230.43 ng PeCDF per kg body weight per day (Fig. 2C). A six-parameter model with common response limits for both chemicals fit the data virtually as well as the eight-parameter model ($F_{2,88} = 1.49$, $p = 0.23$). Taking the chemicals to have common response limits, we estimated $\rho(d_1)$ of Equation 7 directly by a reparameterization of the six-parameter model (Table 3). This estimate of $\rho(d_1)$ was nearly horizontal (Fig. 2D). In fact, eliminating ψ from the six-parameter model barely changed model fit ($F_{1,90} = 0.29$, $p = 0.59$). The constant relative potency estimate resulting from this five-parameter model is well within the simultaneous

FIG. 2. Dose-response and relative potency for TCDD and PeCDF derived from liver A4H activity in rats after 53-week exposure via oral gavage. Enzyme activity was measured as nmol of 4-hydroxyacetanilide formed per min per mg of microsomal protein; dose was measured in ng per kg of body weight per day. Panels (a) TCDD and (b) PeCDF show observed activity for each animal ($\circ$), dose-specific means ($\blacklozenge$), and estimated dose-response curves (solid, eight-parameter model with a separate Hill function for each chemical; dashed, six-parameter model honoring constraint that both chemicals have same response limits; dotted, five-parameter model honoring constraints of equal response limits and constant relative potency). Panel (c) shows two-stage estimate of $\rho(d_1)$ based on estimates from eight-parameter model. Panel (d) shows direct estimates of $\rho(d_1)$ (solid, enforcing power-function model for relative potency; dashed, its 95% simultaneous confidence band; dotted, enforcing constant relative potency).

confidence band for the power-function estimate of $\rho(d_1)$. This five-parameter model also fit well compared with the saturated analysis-of-variance model ($F_{7,84} = 1.19$, $p = 0.32$). We conclude that the relative potency of PeCDF compared with TCDD with respect to liver A4H activity is constant and ~ 0.31 ($= e^{-1.17}$) with 95% confidence limits (0.23, 0.42).

DISCUSSION

When unwarranted, an assumption that relative potency is constant can produce misleading conclusions. Relative potency functions, which describe how relative potency

changes continuously along the paired dose-response trajectories, offer a promising approach for summarizing the potency relationships between chemicals in a flexible way when constancy is in doubt (Dinse and Umbach, 2011; Ritz *et al.*, 2006). Although non-constant relative potency can be described as a function of mean response, of response quantile, or of dose (either test or reference), we focused here on relative potency as a function of dose of the test chemical. Even so, two distinct types of relative potency functions, $\rho(d_1)$ and $\rho^*(d_1)$, deserve consideration. The former function embodies the classical definition of relative potency based on the ratio of doses that yield the same mean response and is appropriate when any differences in response limits are seen as intrinsic properties

TABLE 3

Parameter and SE Estimates for Nested Hill Dose-Response Models Fitted to Liver A4H Activity^a in Rats after 53-Week Exposure to TCDD (Reference Chemical, Subscripted 0) or PeCDF (Test Chemical, Subscripted 1)^b

Common response limits for both chemicals ^c				Constant relative potency	
Parameterization 1		Parameterization 2			
Parameter	Estimate (SE)	Parameter	Estimate (SE)	Parameter	Estimate (SE)
L	0.55 (0.03)	L	0.55 (0.03)	L	0.55 (0.03)
U	3.08 (0.55)	U	3.08 (0.55)	U	2.95 (0.44)
S_0	0.82 (0.17)	S_0	0.82 (0.17)	S_0	0.89 (0.15)
M_0	25.42 (15.59)	M_0	25.42 (15.59)	M_0	21.36 (10.29)
S_1	0.89 (0.16)	η	-1.50 (0.65)	η	-1.17 (0.16)
M_1	75.98 (40.99)	ψ	0.09 (0.17)		
MSE (df) ^d		0.0379 (90)		0.0376 (91)	

^aActivity measured as nmol of 4-hydroxyacetanilide formed per min per mg of microsomal protein.

^bDoses were measured in ng per kg of body weight per day.

^cThe two parameterizations of the six-parameter model are related by $\psi = S_1/S_0 - 1$ and $\eta = \log(M_0) - (S_1/S_0) \log(M_1)$. The second parameterization estimates the relative potency function $\rho(d)$ directly through parameters η and ψ .

^dMean Squared Error, an estimate of the residual variance, and associated degrees of freedom.

of the chemicals themselves. The latter embodies a modified definition of relative potency based on the ratio of $ED_{100\pi}$ values, and this modified definition is only appropriate when any differences in response limits between the chemicals are seen as extrinsic to the chemicals, e.g., resulting from uncontrolled batch-to-batch or laboratory-to-laboratory effects. Such extrinsic differences should not affect conclusions about relative potencies. Accordingly, $\rho^*(d_1)$ inherently rescales responses to the same range, thus appropriately addressing the circumstance where investigators rescale data to a common response range before analysis despite evident differences in response limits. When the response limits for the two chemicals are the same, the classical and modified definitions of relative potency coincide, as do $\rho(d_1)$ and $\rho^*(d_1)$.

Because $\rho(d_1)$ and $\rho^*(d_1)$ express relationships between the two chemicals' dose-response curves, both functions were originally derived and expressed in terms of the dose-response parameters (Dinse and Umbach, 2011; Ritz *et al.*, 2006). In fact, parameter estimates from fitted dose-response curves can be plugged into Equation 3 or Equation 4 to estimate the respective relative potency functions. In our Hill model examples, these parameter estimates could be derived from the eight-parameter model (four parameters for each chemical) or from constrained models that fit the observed data well with fewer parameters. Typically, dose-response models with fewer parameters yield relative potency functions with simpler shapes. We proposed an alternate approach that postulates a power-function model for $\rho(d_1)$ or $\rho^*(d_1)$ and requires specification of a dose-response function or a dose-quantile function, respectively, only for the reference chemical. This approach directly estimates the parameters of the relative potency function, and it amounts to a simple reparameterization of the appropriate dose-response models as expressed in Equations 5 and 6 and implemented in our sample SAS code (Supplementary data).

Where applicable, this alternative approach offers advantages by employing nonlinear regression techniques to estimate a power function for relative potency, as well as simultaneous confidence bands, simply and directly. Restricting attention to settings where both chemicals' dose-response functions have the same form but with possibly different parameter values, we showed that a power-function model for relative potency is compatible with a variety of commonly used dose-response models. That is, a power function for relative potency together with a dose-response model for the reference chemical in a given parametric family induces a dose-response model for the test chemical in the same parametric family. In such families, the power-function model for $\rho^*(d_1)$ is valid whether or not both chemicals have common response limits; on the other hand, a power-function model for $\rho(d_1)$ requires that both chemicals have the same response limits. If interest centers on $\rho(d_1)$ but the dose-response functions have distinct response limits, $\rho(d_1)$ cannot have a power function form; so, estimation must proceed by the two-stage approach.

In conjunction with a dose-response model for the reference chemical, a two-parameter power-function model for relative potency—whether $\rho(d_1)$ or $\rho^*(d_1)$ —always fits the joint dose-response data for both chemicals at least as well as the one-parameter model of constant relative potency. On the other hand, use of the power-function model for $\rho(d_1)$ with any compatible dose-response model forces the test chemical to have the same lower and upper response limits as the reference chemical. Consequently, fitting a power function for $\rho(d_1)$ can degrade fit by constraining response limits to be equal when they are not. The problem arises, in part, because the power-function model is monotone and defined over the entire dose range whereas, when response limits differ between chemicals, $\rho(d_1)$ may change directions more than once and be defined only over a restricted range of doses (Dinse and Umbach, 2011). A power-function model for

$\rho^*(d_1)$, however, will not degrade fit even when response limits differ between chemicals. It incorporates a modified definition of relative potency that is constructed to recover the classical concept in the face of differences in response limits—but the construction is meaningful only if those differences are not due to the chemicals themselves.

Using EROD and A4H enzyme activity data from NTP bioassays, we illustrated the utility of directly estimating a relative potency function. For liver EROD activity, we found that separate Hill models for each chemical fit adequately, but that no simpler models were consistent with the observations. In particular, PeCDF and TCDD appeared to have distinct response limits. If we view the difference in response limits as intrinsic to the chemicals, the relative potency function $\rho(d_1)$, embodying the classical definition of relative potency, is more complex than our proposed power-function model and must be estimated in two stages from all eight parameters of the fitted Hill models. This relative potency function was defined on an interval of doses and exhibited strong curvature and vertical asymptotes as it approached the limits of that range. Alternatively, if we view the difference in response limits as extrinsic to the chemicals, the relative potency function $\rho^*(d_1)$ can be estimated explicitly via a reparameterization of the eight-parameter model. This relative potency function was non-constant and was defined for any dose; it suggested that the potency of PeCDF became smaller relative to TCDD as dose increased.

For liver A4H activity, we found that the dose-response relationships were adequately modeled by two Hill functions constrained to have the same lower and same upper response limits (six-parameter model). When response limits are the same for both chemicals, $\rho(d_1)$ and $\rho^*(d_1)$ are identical and can be estimated explicitly by a reparameterization of the six-parameter dose-response model. The resulting relative potency function was nearly constant and, in fact, fit no better than a model enforcing constant relative potency.

Relative potency functions offer a promising way to relax the assumption of constant relative potency while still quantifying the potency relationships among chemicals. Instead of estimating relative potency functions by plugging parameter estimates from fitted dose-response functions into appropriate expressions, we proposed instead modeling relative potency as a function of the test chemical's dose with a power function and estimating its parameters directly as part of the dose-response fitting process. The proposed approach is widely applicable, but not universally so. In our examples, the power-function form proved convenient and amenable to use with $\rho^*(d_1)$ and $\rho(d_1)$, though its use with the latter requires both chemicals to have the same response limits.

SUPPLEMENTARY DATA

Supplementary data are available online at <http://toxsci.oxfordjournals.org/>.

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