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Characterizing Non-Constant Relative Potency

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Abstract

Relative potency plays an important role in toxicology. Estimates of relative potency are used to rank chemicals by their effects, to calculate equivalent doses of test chemicals compared to a standard, and to weight contributions of constituent chemicals when evaluating mixtures. Typically relative potency is characterized by a constant dilution factor, even when non-similar dose-response curves indicate that constancy is inappropriate. Improperly regarding relative potency as constant may distort conclusions and potentially mislead investigators or policymakers. We consider a more general approach that allows relative potency to vary as a function of dose, response, or response quantile. Distinct functions can be defined, each generalizing different but equivalent descriptions of constant relative potency. When two chemicals have identical response limits, these functions all carry fundamentally equivalent information; otherwise, relative potency as a function of response quantile is distinct and embodies a modified definition of relative potency. Which definition is preferable depends on whether one views any differences in response limits as intrinsic to the chemicals or as extrinsic, arising from idiosyncrasies of data sources. We illustrate these ideas with constructed examples and real data. Relative potency functions offer a unified and principled description of relative potency for non-similar dose-response curves.

Keywords

Dose conversion; Dose response; ED_{50} ; Hill model; Non-similar dose-response curves; Relative potency factor

1. Introduction

Toxicologists use estimates of relative potency for ranking chemicals (*e.g.*, Glass *et al*, 1991) and for dose-conversion analyses that calculate the equivalent dose of one chemical that produces the same response as a specific dose of another (*e.g.*, Putzrath, 1997). They also combine separate estimates of relative potency, from different studies and endpoints, to determine a single toxic equivalency factor (TEF) (*e.g.*, Van den Berg *et al*, 2006). The

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Supplementary Data

The liver EROD activity data used in our example are available online as an Excel spreadsheet.

National Toxicology Program may soon use relative potency estimates in prioritizing chemicals via high throughput screening.

Relative potency of a test compound compared to a reference compound is typically thought of as a ratio of doses (reference divided by test) that produce the same mean response for a given endpoint. The notion that relative potency should be constant has roots in analytical dilution assays where all preparations are regarded as dilutions of a standard preparation with an inert diluent (Finney, 1965). In that context, the ratio of doses producing the same mean response should be identical at every response level. Consequently, when dose-response curves for such preparations are plotted on a log-dose axis, the curves should be identical up to a horizontal shift (Figure 1). Even when preparations are not simple dilutions of a single active compound, there may be biological reasons (e.g., a common mechanism of toxicity) to expect them to behave as though they were and, thus, to have constant relative potencies. Chemicals with constant relative potency are said to have 'similar' dose-response curves (*i.e.*, equal up to a horizontal shift on the log-dose axis). Their relative potency is characterized by a single dilution (or concentration) factor, a feature that simplifies ranking chemicals and calculating equivalent doses.

Researchers have long recognized that constant relative potency is not always reasonable because comparative assays often yield dose-response curves that differ in ways other than a horizontal shift (Cornfield, 1964; Cox and Leaverton, 1966; Rodbard, 1974; De Lean *et al*, 1978; Guardabasso *et al*, 1987; Guardabasso *et al*, 1988). When dose-response curves are not similar, regarding them as similar and estimating relative potency accordingly may distort conclusions.

For example, consider the activity of a liver enzyme in response to TCDD, PCB126, PeCDF and their TEF-based mixture (National Toxicology Program, 2006a–d). For illustration purposes, we regard the mixture as a fourth chemical. When a separate dose-response model is fit to each chemical and the ratio of median effective doses (ED_{50} s) is used to assess relative potency despite non-similarity (Figure 2a), the estimated potencies relative to TCDD suggest ranking the chemicals (from most to least potent) as: TCDD > Mixture > PCB126 > PeCDF. When the dose-response models are constrained to enforce similarity (Figure 2b), the constant relative potencies, determined as ratios of revised ED_{50} s, suggest the ranking: Mixture > TCDD > PeCDF > PCB126. These rankings differ, in part, because the chemicals vary substantially in their apparent upper limits of response. Both rankings indicate that TCDD and the mixture are more potent than PeCDF and PCB126, but they give opposite results for TCDD versus the mixture and for PeCDF versus PCB126. In both the constrained and unconstrained analyses, the mixture's fitted curve lies above TCDD's at all doses (Figure 2), indicating that the mixture is the more potent and, in turn, demonstrating that the use of ED_{50} s for estimating relative potency is problematic when dose-response curves are not similar. The unconstrained dose-response curves for TCDD and PeCDF cross twice, suggesting that their relative potency changes across response levels and doses; consequently, using any single value of relative potency for ranking or dose conversion would be an oversimplification.

Despite recognition of these issues, approaches to analysis that explicitly allow non-constant relative potencies are rare. Cornfield (1964) relaxed the similarity constraint, but he assumed that response was a linear function of log-dose (see, also, Cox and Leaverton, 1966); typically linearity is reasonable for only a portion of the dose-response curve. Under a more general dose-response model, DeVito *et al* (2000) assumed relative potency was constant for doses below some point and otherwise was linear in the reciprocal of dose. Several researchers suggested accommodating non-similar dose-response curves by simply reporting estimates of relative potency at a few selected doses (Putzrath, 1997; Villeneuve *et al*,

2000). Recently, Ritz *et al* (2006) formulated relative potency as a function of response. These proposals notwithstanding, toxicologists often estimate relative potency by the ratio of ED_{50} s despite non-similarity of the dose-response curves (Rodbard and Frazier, 1975; Villeneuve *et al*, 2000).

This article builds on previous proposals for quantifying relative potency for non-similar dose-response curves. We define functions that describe relative potency as depending on dose, response, or response quantile. In general, the ratio of doses used to define relative potency depends on where along the dose-response curves the ratio is taken; these functions reflect that dependence. We develop our proposals conceptually and graphically, describing their utility and limitations, and we defer technical details to an appendix. We postpone a full discussion of the statistical analysis of relative potency functions as beyond our present scope. We illustrate our proposals with the NTP enzyme activity data introduced earlier.

2. Background

2.1 Modeling Dose-Response

A dose-response model expresses the mean (or average) response as a mathematical function of dose. For a given endpoint, we use the notation $f(d;\theta)$ to represent the mean response elicited by dose d of the chemical of interest, where f is a monotone function of d that depends on a vector θ of unknown parameters. We consider dose-response curves where the mean response increases from a lower limit, L , at a dose value of zero ($d = 0$) to an upper limit, U , at an infinite dose ($d = \infty$). If the opposite is true, so that the curve decreases from an upper limit, the ideas that we describe still apply after reversing the definitions of L and U .

Various functions can be used for f . A sigmoid function commonly used for dose-response relationships is the Hill (1910) model:

$$f(d;\theta) = L + (U - L)d^S / (M^S + d^S). \quad (1)$$

The parameter vector is $\theta = (L, U, S, M)$, where S governs the shape (or steepness) of the dose-response curve and M represents the median effective dose (ED_{50}). All dose-response curves in our figures are based on Hill models, plotted with dose on a logarithmic scale, though our relative potency concepts are applicable to any pair of monotone dose-response models.

2.2 Similar Dose-Response Curves

Suppose that a reference chemical C_0 and a test chemical C_I have similar dose-response curves for the endpoint of interest. Because similar dose-response curves are identical up to a constant horizontal shift, they have the same functional form (f) and same response limits but possibly distinct values for other parameters. Similar Hill-model curves, for example, have the same response limits and shapes ($L_0 = L_1$, $U_0 = U_1$, $S_0 = S_1$) but their ED_{50} s can differ ($M_0 \neq M_1$).

For any monotone function f , similarity implies that (Finney, 1965):

$$f(d;\theta_1) = f(\rho d;\theta_0) \text{ for every dose } d > 0; \quad (2)$$

ρ is a constant representing the relative potency of C_I compared to C_0 , and θ_1 and θ_0 are dose-response parameter vectors for the two chemicals, respectively. In other words, if dose

d_0 of C_0 and dose d_1 of C_1 produce identical mean responses, then setting $d = d_1$ in equation (2) implies that $d_0 = \rho d_1$, or that $\rho = d_0 / d_1$, for any mean response. Thus, for similar dose-response curves, relative potency ρ is defined as the ratio of doses d_0 and d_1 that produce the same mean response level, say μ , and ρ is the same for any choice of μ . That is, for similar dose-response curves, relative potency does not change with mean response (Figure 1). In particular, if one chooses a mean response halfway between the lower and upper limits, the relative potency is the ratio of ED_{50} s (of course, one could choose any fraction π between 0 and 1, take the ratio of the $ED_{100\pi}$ s for the two chemicals, and obtain the same value of ρ). Equipotent chemicals have $\rho = 1$.

3. Relative Potency Functions

3.1 Concepts

When similar dose-response curves are plotted with dose on a logarithmic scale, the magnitude of the logarithm of ρ equals the horizontal distance between the curves at any mean response level μ ; and the sign of the logarithm depends on the direction from the test chemical's curve to the reference chemical's curve (left being negative and corresponding to relative potencies less than 1) (Figure 1). (Relative potency is not defined for values of μ below L or above U .) For non-similar dose-response curves, this signed horizontal distance varies with mean response μ (Figure 3a). Accordingly, one can express how the value of ρ changes with μ using the notation of mathematical functions (Ritz *et al*, 2006). We call the corresponding relative potency function $\rho_\mu(\mu)$ to reflect the dependence of ρ on μ ; in addition to μ , it depends on the parameters θ_0 and θ_1 of the dose-response model (Appendix). The relative potency function $\rho_\mu(\mu)$ relates each allowable value of μ to the corresponding value of ρ , that is, to the antilog of the signed horizontal arrow length (which is equal to the μ -specific value of the ratio d_0 / d_1). For similar dose-response curves, $\rho_\mu(\mu)$ is a constant function.

Because each value of μ corresponds to a specific value of either d_1 or d_0 , relative potency can be indexed by either of these doses instead of by mean response. In terms of Figures 1 or 3a, besides its vertical position, any arrow can be indexed by the horizontal position on the log dose axis where it starts (its tail) or ends (its head), positions that correspond to d_1 or d_0 , respectively. Accordingly, we define the relative potency function $\rho_{d_1}(d_1)$ as relating a value of d_1 to the corresponding value of ρ ; and we define $\rho_{d_0}(d_0)$ analogously.

When the dose-response curves for C_0 and C_1 have both upper and lower response limits in common (whether the curves are similar or not), any mean response μ can also be indexed by its relative position between L and U , namely, by $\pi = (\mu - L) / (U - L)$. Here, π is a response quantile that represents the fraction of the distance between the lower and upper response limits. As μ varies from L to U , the corresponding π varies from 0 to 1. Because both chemicals have the same response limits, each value of π corresponds to a common value of μ for both chemicals. Consequently, we can define the relative potency function $\rho_\pi(\pi)$ as relating a value of π to the corresponding relative potency; $\rho_\pi(\pi)$ is given by the ratio of the $ED_{100\pi}$ s for the two chemicals (Figure 1). The situation changes when C_0 and C_1 have different lower or upper response limits. A single value of π now corresponds to a distinct value of μ for each chemical (Figure 3b). Nevertheless, we retain the definition of $\rho_\pi(\pi)$ as the ratio of $ED_{100\pi}$ s for C_0 and C_1 , and thereby modify the usual definition of relative potency by using a ratio d_0 / d_1 where the doses correspond to different values of μ . For curves with different response limits, this definition corresponds graphically to the antilog of the horizontal component of the vector that connects a given response quantile π for the test chemical to the same quantile for the reference chemical (Figure 3b).

The relative potency function $\rho_{\pi}(\pi)$ embodies the usual concept of relative potency only when the response limits for the two chemicals are the same; only then will a specified value of π represent the same value of μ for both chemicals so that the resulting concept of relative potency coincides with the usual concept. When the response limits differ, one value of π corresponds to different values of μ for each chemical (despite representing the same response quantile), thereby modifying the usual concept of relative potency. This modified concept is closely related to the idea of standardizing response variables by shifting and rescaling them to have a range from 0 to 1. A mathematical development of the relative potency functions $\rho_{\mu}(\mu)$, $\rho_{d_0}(d_0)$, $\rho_{d_1}(d_1)$ and $\rho_{\pi}(\pi)$ for both general dose-response models and Hill models is available in the Appendix.

3.2 Three Illustrative Examples

We illustrate some characteristics of the different relative potency functions using three examples of dose-response curves for a reference chemical C_0 and a test chemical C_I (Figure 4). By plotting relative potency on a logarithmic scale, we actually visualize log relative potency functions. These examples are based on a pair of Hill models: each example uses the same model for the reference chemical C_0 but a different model for the test chemical C_I .

First, we consider two chemicals whose dose-response functions have the same response limits ($L_0 = L_1$ and $U_0 = U_1$) but differ in shape ($S_0 > S_1$) and ED_{50} ($M_0 < M_1$) (Figure 4, row a). The dose-response curve for C_0 is to the right of the curve for C_I up to a dose of ~ 3.16 ; beyond that, the curve for C_0 lies to the left of that for C_I , with the horizontal distance increasing as the dose of either chemical increases (we use d_i as shorthand for either d_0 or d_1). This description corresponds to relative potencies exceeding 1 up to dose 3.16 (correspondingly, $\mu = 21.84$ or $\pi = 0.03$) and falling ever farther below 1 as d_i (or μ or π) continues to increase. All relative potency functions reflect that description (Figure 4, row a), although the respective curves do differ qualitatively. Because both chemicals have the same response limits, all curves span precisely the same set of relative potency values; the different shapes simply reflect differences imposed by the arguments (μ , d_i or π) of the function. For example, μ must lie between 20 and 80, so $\rho_{\mu}(\mu)$ is defined only for μ between those values; similarly, π must lie between 0 and 1. On the other hand, because μ is between 20 and 80 for any positive dose, $\rho_{d_i}(d_i)$ is defined for any positive d_i . In fact, with both dose and relative potency plotted on a log scale, $\rho_{d_i}(d_i)$ for Hill-model dose-response functions must be a straight line whenever the two chemicals have the same lower and upper response limits, regardless of their shape and ED_{50} parameters (Appendix). In this example, a fixed relative potency based on the ratio of ED_{50} s ($M_0/M_1 = 0.1$) suggests that C_I is less potent than C_0 by a constant amount, a potentially misleading conclusion in light of Figure 4, row a.

Next, consider two chemicals with different lower and upper limits of mean response ($L_0 > L_1$ and $U_0 > U_1$) and different ED_{50} s ($M_0 > M_1$) but the same shape parameters ($S_0 = S_1$) (Figure 4, row b). We selected parameter values so that the dose-response curve for C_0 is to the left of the curve for C_I at all doses. The minimal horizontal separation from C_I to C_0 is -0.12 (equivalent to a relative potency of $\exp(-0.12) = 0.89$) at $\mu = 42.5$, $d_0 = 8.43$ or $d_1 = 9.49$; and it increases in magnitude (but remains negative) as doses or responses increase or decrease from those values. The horizontal separation is infinite (negative) for μ between 5 and 20 (which corresponds to $d_0 = 0$ or $d_1 < 5.55$) or for μ between 65 and 80 (which corresponds to $d_0 > 14.42$ or $d_1 = \infty$). This description in terms of negative horizontal separations corresponds to relative potencies that are always less than 1 and tend to get ever closer to zero as either dose or response approaches its limits. The relative potency functions $\rho_{\mu}(\mu)$ and $\rho_{d_i}(d_i)$ reflect these descriptions (Figure 4, row b); in particular, relative potency is everywhere less than 1 and goes to 0 (its log goes to $-\infty$) as either μ or d_i approaches its

limits. These relative potency functions span the same set of relative potency values and, again, differences in the appearance of the curves reflect restrictions on their respective arguments. On the other hand, $\rho_{\pi}(\pi)$ is remarkably different from the other relative potency functions. It is constant with a value of 1.25 ($= M_0/M_1$), which suggests the test chemical is more potent than the reference, rather than less potent as the other relative potency functions and the two dose-response curves indicate. This distinctness of $\rho_{\pi}(\pi)$ arises because, whenever chemicals differ in their response limits, $\rho_{\pi}(\pi)$ embodies a modified definition of relative potency. In this example, uncritical use of the ED_{50} ratio, or any $ED_{100\pi}$ ratio, to assess relative potency might be seriously misleading.

Our third example considers two chemicals with the same ED_{50} values but different shape parameters ($S_0 > S_1$) and different response limits such that those for C_0 fall between those for C_1 ($L_1 < L_0 < U_0 < U_1$) (Figure 4, row c). The dose-response curves cross three times and the more potent chemical switches with each crossing. Whenever the dose-response curves cross, the relative potency functions $\rho_{\mu}(\mu)$ and $\rho_{d_i}(d_i)$ cross the equipotent value 1, precisely tracking which chemical is locally more potent. In contrast, $\rho_{\pi}(\pi)$ behaves differently: it crosses 1 only once at $\pi = 0.5$. In this example, none of the relative potency functions is consistent with a constant relative potency equal to 1, the ratio of ED_{50} s.

Readers interested in visualizing dose-response curves and relative potency functions for different combinations of Hill model parameters can access a JAVA program called Hill Viewer at <http://www.niehs.nih.gov/research/atniehs/labs/bb/resources.cfm>.

3.3 Choice of Relative Potency Function

With multiple relative potency functions available, the issue of how to choose among them arises. Generally speaking, if both chemicals have the same response limits, all these functions span the same range of relative potency values and carry equivalent information; the only difference among the graphs of the functions is how the horizontal axis is stretched or deformed as the function's argument changes from mean response μ to dose d_0 of C_0 , or to dose d_1 of C_1 , or to response quantile π . On the other hand, when the upper or lower response limits differ between the chemicals, $\rho_{\pi}(\pi)$ is distinct from $\rho_{\mu}(\mu)$, $\rho_{d_0}(d_0)$ and $\rho_{d_1}(d_1)$, though these latter three relative potency functions still carry fundamentally equivalent information.

Because $\rho_{\mu}(\mu)$, $\rho_{d_0}(d_0)$ and $\rho_{d_1}(d_1)$ represent an extension of the usual definition of relative potency based on the horizontal shift in the dose-response curves, whereas $\rho_{\pi}(\pi)$ represents a slight modification of that definition, care must be taken in choosing between them. The fundamental issue involves the source or nature of any differences between the upper or lower limits of the dose-response curves for C_0 and C_1 . If these differences are intrinsic to the chemicals for the response under test, we argue that this intrinsic feature should be taken into account when considering relative potency, and that $\rho_{\mu}(\mu)$, $\rho_{d_0}(d_0)$ or $\rho_{d_1}(d_1)$ should be preferred over $\rho_{\pi}(\pi)$. For example, suppose one were studying the response of a natural population to two pesticides and a subset of the population was essentially immune to one of the pesticides. As the dose increased, the proportion killed by one pesticide would be maximal at some value less than 100%, whereas the other would achieve 100%. In that situation, the differences in response limits seem intrinsic to evaluating the relative potency of the pesticides in this population.

On the other hand, if differences in response limits are extrinsic to the chemicals for the response under test, those differences should not influence relative potency and $\rho_{\pi}(\pi)$, which effectively rescales the responses to the same range, should be preferred. For example, if the dose-response experiment for each chemical was conducted separately with respect to some factor that should be irrelevant to relative potency itself (e.g., in two

different labs or on separate cultures of cells grown days apart within the same lab), the difference in upper or lower response limits might not be related to the chemicals themselves but instead to different experimental conditions. In that situation, an investigator might justifiably believe that differences in response limits were extrinsic to the relative potency of the chemicals. The function $\rho_{\pi}(\pi)$ honors the extrinsic nature of the response limits by, in effect, enforcing the idea that the mean response, all else being equal, would have the same range for both chemicals.

If differences in the upper and lower limits of mean response are regarded as intrinsic, the choice between $\rho_{\mu}(\mu)$ and one of the $\rho_{d_i}(d_i)$, although less critical, remains. Because these functions carry essentially the same relative-potency information, just graphed against a different variable, the functions may be used somewhat interchangeably to get a picture of non-constant relative potency. Because the range of μ for which $\rho_{\mu}(\mu)$ is positive and finite is circumscribed by $\max(L_0, L_1) < \mu < \min(U_0, U_1)$ (Appendix), $\rho_{\mu}(\mu)$ is often more convenient for quantitative calculations, as indicated in the following subsection. For dose-conversion calculations, however, one of the $\rho_{d_i}(d_i)$ may be more convenient because they map relative potency directly to dose levels. When ρ is constant, the dose d_0 of C_0 that is equivalent to a specified dose d_1 of C_1 is given by $d_0 = \rho d_1$. To make the same conversion using a relative potency function, simply substitute $\rho_{d_1}(d_1)$ for ρ . Analogously, conversion of a specified dose d_0 of C_0 to the equivalent dose d_1 of C_1 is achieved by setting $d_1 = d_0/\rho_{d_0}(d_0)$. Features of relative potency functions with guidelines for choosing among them are summarized in Table 1.

3.4 Summaries of Relative Potency Functions

Relative potency functions were conceived as a way to address a common problem: the desire to characterize potency for pairs of chemicals whose dose-response curves are not strictly similar and thus have non-constant relative potency. In that setting, a single summary value such as the ratio of ED_{50} s is potentially misleading. Consequently, a principal use of relative potency functions will be graphical, displaying how the relative potency of two chemicals changes in the absence of similarity. As valuable as graphs are for this purpose, however, simple quantitative summaries and other numerical quantities derived from relative potency functions will likely be useful. We illustrate several ideas using $\rho_{\mu}(\mu)$, though the concepts apply to the other relative potency functions as well.

For example, if a relative potency function is not constant but does not intersect the line of equipotency ($\rho = 1$), then one chemical is everywhere more potent than the other and having a single numerical summary of that relationship might be useful. One possible summary value that can be derived from a relative potency function is the closest value of $\rho_{\mu}(\mu)$ to the null value of 1, denoted here by ρ_{μ} . Two chemicals are equipotent for at least one response level if $\rho_{\mu} = 1$; otherwise, ρ_{μ} serves as an index of relative potency. Whenever the upper and lower limits of response are the same for both chemicals, the value of ρ_{μ} equals the values of ρ_{d_0} , ρ_{d_1} and ρ_{π} calculated using the other relative potency functions; for the Hill model, each ρ is 1 if $S_0 \neq S_1$ (see, e.g., Figure 4, rows a and c) and is M_0/M_1 if $S_0 = S_1$ (see, e.g., Figure 4, row b). Whenever the response limits differ for the two chemicals, the values of ρ_{μ} , ρ_{d_0} and ρ_{d_1} will be equal but can differ from that of ρ_{π} . A disadvantage of ρ_{μ} and its analogs is that any relative potency function that intersects the equipotency line has exactly the same summary value, namely, 1.

An alternative summary that addresses this difficulty is an average, although problems arise when $\rho_{\mu}(\mu)$ is infinite or undefined for some values of μ . We suggest using the geometric mean of $\rho_{\mu}(\mu)$ over a specified interval (a, b) of finite response levels. This geometric mean, denoted $\rho_{\mu}^{(a,b)}$, is calculated as the anti-log of a definite integral of the log relative potency

function (Appendix). A drawback is that the geometric mean relative potency can differ depending on which relative potency function is used for the calculation, reflecting rules for change of variables in integration. Interestingly, for Hill dose-response curves, the geometric mean of $\rho_\pi(\pi)$ for any interval (a,b) centered on $\pi = 0.5$ always equals the ratio of the ED_{50} s (Appendix), perhaps providing a rationale for favoring the geometric mean over the arithmetic mean when averaging relative potency functions. Of course, no single number can fully describe a non-constant relative potency function, so care must be taken when interpreting such summaries.

Another potentially useful summary is the set of mean response levels where relative potency lies within a specified interval (l,m) , that is, the set $D_\mu^{(l,m)} = \{\mu: l < \rho_\mu(\mu) < m\}$. For example, if we are interested in the range of responses where one chemical is more potent than another, the set $D_\mu^{(1,\infty)}$ or its complement $D_\mu^{(0,1)}$ would be useful. Alternatively, if we want to determine a range of responses over which the potencies of two chemicals differ by at most some small amount δ , which corresponds to an ‘indifference zone’ or an ‘equivalence region’ near equipotency, we could use $D_\mu^{(1-\delta, 1+\delta)}$ for an appropriately chosen δ .

3.5 Multiple Relative Potency Functions

Often, investigators want to compare several test chemicals to the same reference chemical. Consider two test chemicals, C_1 and C_2 , and a common reference chemical C_0 . Because the relative potency function of C_2 relative to C_1 can be expressed as the quotient of their separate relative potency functions with respect to C_0 (with attention to the range over which the quotient is defined), information about the relative potency of C_2 relative to C_1 can be gleaned from the separate curves. Non-intersecting curves indicate that one chemical is consistently more potent than the other and thus the chemicals can be ranked with respect to toxicity. On the other hand, if the relative potency functions cross, the ranking will vary with mean response, or dose, or response quantile so that comparisons that rely on a single summary value from each relative potency function must be interpreted with caution.

4. Application to Enzyme Activity Data

The U.S. National Toxicology Program (NTP) recently evaluated the relative potency of dioxin-like compounds with respect to toxicity and carcinogenicity endpoints (NTP, 2006a-d). They studied 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 3,3',4,4',5-pentachlorobiphenyl (PCB126), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and a TEF-based mixture of the three. We used a subset of these data (Online Supplement) to illustrate several concepts introduced earlier. Specifically, we focused on the activity of cytochrome P450 1A1-associated 7-ethoxyresorufin-*O*-deethylase (EROD) as measured in liver tissue of female Harlan Sprague-Dawley rats treated by oral gavage for 53 weeks (Toyoshiba *et al*, 2004). There were 8 rats per dose for each chemical, though dose levels and numbers of doses varied across chemicals. To illustrate our ideas, we treated the mixture as a fourth chemical, ignoring its composition.

We used SAS Proc NLIN (version 9.00, SAS Institute Inc., Cary, NC, USA) to fit Hill models to log-transformed EROD activity using unweighted least squares. Initially, we fit an unrestricted model, with four parameters for each of the four chemicals (Table 2). Individual Hill models appeared to fit the data well, as evidenced by the proximity of the estimated dose-response curves to the dose-specific mean EROD levels (Figure 5).

We assessed similarity by allowing each dose-response curve to have a distinct ED_{50} but restricting the lower response limits, upper response limits and shape parameters to be the same across chemicals (Table 2). Chemical-specific dose-response curves estimated after

enforcing similarity did not fit the data as well as the unrestricted curves (Figure 5); the hypothesis that all four chemicals had similar dose-response curves with respect to EROD activity was rejected (F test of fit, $p < 10^{-15}$). The poor fit under full similarity is likely due to the PeCDF responses not reaching a plateau, which led to a large (and variable) estimate of the upper response limit (Table 2). When considered in pairs, TCDD and PCB126 appear to have similar dose-response curves ($p = 0.65$), but TCDD and PeCDF ($p < 0.001$) and TCDD and the mixture ($p = 0.02$) do not.

Following convention, we treated TCDD as the reference and estimated the relative potency functions of the other chemicals compared to TCDD. In accord with the preceding results, the Hill models for PeCDF and the mixture were unrestricted, but those for TCDD and PCB126 were constrained to produce similar dose-response curves (Table 2, Figure 6). We estimated relative potency functions by substituting the parameter estimates from the fitted Hill models into the appropriate formulae (Appendix).

Because TCDD and PCB126 were forced to have similar dose-response curves, relative potency functions for those chemicals were constant (Figures 6b, 6c and 6d) and equal to the ratio of their ED_{50} s ($\hat{\rho} = 0.19$, 95% CI: 0.16, 0.22), indicating that PCB126 is less potent than TCDD. The chemicals allowed to have non-similar dose-response curves had estimated relative potency functions that deviated substantially from constant functions.

If one considers the differences between the lower and upper response limits for these chemicals as intrinsic to the comparison, attention should focus on $\rho_{\mu}(\mu)$ or one of the $\rho_{d_i}(d_i)$ (Figure 6 displays $\rho_{d_0}(d_0)$ but not $\rho_{d_1}(d_1)$). For the mixture compared to TCDD, the estimated relative potency functions $\hat{\rho}_{\mu}(\mu)$ and $\hat{\rho}_{d_0}(d_0)$, exceeded the null value of 1 for every μ and d_0 , respectively, suggesting the mixture is more potent than TCDD. In fact, the value of either $\hat{\rho}_{\mu}(\mu)$ or $\hat{\rho}_{d_0}(d_0)$ closest to 1 was $\rho_{\mu} = \rho_{d_0} = 1.73$, which occurred at $\mu = 1115$ and $d_0 = 7.67$, respectively. On the other hand, for PeCDF relative to TCDD, $\hat{\rho}_{\mu}(\mu)$ and $\hat{\rho}_{d_0}(d_0)$ each crossed the equipotency line ($\rho = 1$) at two places ($\mu = 159$ and 1886; $d_0 = 0.88$ and 58.15), suggesting PeCDF could be more potent or less potent than TCDD, depending on the mean response or dose considered. The geometric mean values of $\hat{\rho}_{\mu}(\mu)$ were $\check{\rho}_{\mu} = 0.19$ over $34.5 < \mu < 1984$, $\check{\rho}_{\mu} = 0.86$ over $77.2 < \mu < 1984$, and $\check{\rho}_{\mu} = 3.79$ over $70.9 < \mu < 1984$ for PCB126, PeCDF and the mixture, respectively, relative to TCDD. The upper limit of integration was the same in each case, as the reference TCDD had the lowest value of U , but the lower limit of integration varied, as TCDD did not have the highest value of L . Because $\hat{\rho}_{\mu}(\mu)$ and $\hat{\rho}_{d_0}(d_0)$ for the three test chemicals cross each other only near edges of the range of integration, the ordering of these averages agrees with the general appearance of the curves, with the relative potency curve for the mixture lying above that for PeCDF, which in turn lies above that for PCB126.

On the other hand, if one considers the differences between the lower and upper response limits as extrinsic to the comparison, attention should focus on $\rho_{\pi}(\pi)$. Only for PCB126 does $\hat{\rho}_{\pi}(\pi)$ fail to cross the equipotency line, though the crossing point for PeCDF at $\pi = 0.001$ is near the edge of the range. For either PCB126 or PeCDF, $\hat{\rho}_{\pi}(\pi)$ suggests that the respective compounds are less potent than TCDD, whereas $\hat{\rho}_{\pi}(\pi)$ for the mixture crosses the equipotency line at $\pi = 0.2$, with a negative slope, suggesting that the mixture is more potent than TCDD at low quantiles of response but less potent at higher quantiles. The average values of $\hat{\rho}_{\pi}(\pi)$ over the interval $0 < \pi < 1$ were $\check{\rho}_{\pi} = 0.19$, 0.05, and 0.76 for PCB126, PeCDF and the mixture, respectively, relative to TCDD, in qualitative agreement with the ordering suggested by the curves themselves. The qualitative ordering of the chemicals based on $\hat{\rho}_{\pi}(\pi)$ differs from that based on $\hat{\rho}_{\mu}(\mu)$ or $\hat{\rho}_{d_0}(d_0)$, so the issue of whether evident differences in the response limits are intrinsic or extrinsic to the potency comparison has a definite impact.

5. Discussion

The relative potency of chemicals is a fundamental concern of toxicology. The usual approach is to report a single constant value for relative potency. This approach is sound only when the chemicals have similar dose-response curves. Investigators have, however, long recognized that the ideal of similarity is not always realized in comparative assays. Some analysts simply enforce similarity in fitting curves so that relative potency is constant and estimated as the ED_{50} ratio for the similar curves. Others estimate relative potency as the ED_{50} ratio without enforcing similarity even for non-similar curves (Rodbard and Frazier, 1975; Villeneuve *et al*, 2000). Still other analysts 'standardize' the data for each chemical by subtracting a data-based estimate of L from every observed response and then dividing by a data-based estimate of the response range $U - L$ (e.g., Streibig *et al*, 1995). None of these approaches are completely satisfactory for chemicals with non-similar dose-response curves. Enforcing similarity when it does not hold can misrepresent dose-response relationships. Using a single relative potency value can be misleading when relative potency is not constant, especially if dose-response curves cross. Standardizing the data affects statistical fitting procedures by inducing correlations that are unaccounted for in the usual analyses.

To address some of these issues, Ritz *et al* (2006) expressed relative potency as a function of mean response ($\rho_{\mu}(\mu)$ in our notation). We elaborated on this extension of the concept of relative potency from a constant to a function. Regarded as a constant, relative potency represents a global comparison of potency between two chemicals; regarded as a function, it captures a changing local characterization of the potency relation between the chemicals. When chemicals have similar dose-response curves, a global summary of relative potency is appropriate and its graph would be a horizontal line, that is, a constant function of mean response, of the dose of either the reference or test chemical, or of response quantile. For non-constant relative potency, one needs a convenient descriptor for how relative potency changes. To describe changing relative potency, then, one naturally considers indexing it to those same quantities: mean response, dose, and response quantile. Using hypothetical examples and real data, we illustrated pairs of dose-response curves whose relative potency functions depart from a flat line and indicated some consequences of ignoring such departures. For simplicity, we used the same functional form, the Hill model, for both chemicals; however, the concepts we introduced would apply even if the dose-response models had distinct functional forms. Our hypothetical examples support our contention that, when chemicals do not have similar dose-response curves, use of a constant value for relative potency (typically based on a ratio of ED_{50} s) may be seriously misleading. We reanalyzed NTP EROD-activity data to show the potential value of estimating relative potency by a function rather than by ED_{50} ratios alone or by forcing dose-response curves to be similar.

When two dose-response functions have different upper or lower limits of response, the relative potency functions $\rho_{\mu}(\mu)$, $\rho_{d_0}(d_0)$ and $\rho_{d_1}(d_1)$ express a similar concept of relative potency but one distinct from that expressed by $\rho_{\pi}(\pi)$. Which of these two concepts makes more sense in any given situation depends crucially on whether the discrepancies in response limits between the chemicals are regarded as intrinsic to the chemicals themselves for that response or as extrinsic, deriving instead from sources like the dose-response experiments being conducted on different days or in different labs or on different strains of organism. If the source of discrepancies in response limits is extrinsic, then arguably $\rho_{\pi}(\pi)$ is the more suitable relative potency function because it ignores those discrepancies. It is conceptually related to data standardization (Appendix) without the inherent drawbacks. On the other hand, if the source of discrepancies in response limits is intrinsic, then one of the other relative potency functions should be preferred because they honor unequal response

limits. They also maintain the classical concept of relative potency but apply it locally rather than globally.

Our presentation has stressed the concept of relative potency functions, describing what they are and suggesting ways that they might be used. We have treated them as mathematical objects, not as estimates from data. Consequently, we have not presented methods for the statistical analysis of these functions. For example, users will want to estimate not only the functions themselves, as we have done in our example, but also to derive confidence bands for the curves or to test hypotheses about them. In addition, statistical inference procedures will be needed for our proposed summary measures. Availability of valid and efficient inference procedures will enhance the utility of relative potency functions. Because the relative potency functions are defined by the parameters of dose-response models, confidence limits and statistical tests about relative potency functions and related quantities follow, in principle, from information about the dose-response parameters themselves. After the parameters of the dose-response curves and their variance-covariance matrix are estimated using standard statistical methods, those estimates can be translated into inferences about a relative potency function. We are currently investigating such inference procedures.

A toxic equivalency factor (TEF) summarizes the potency of one chemical relative to another by a single number that is independent not only of dose and response but also of endpoint, laboratory, and other sources of variation. TEFs are commonly derived for classes of chemicals that share a common mechanism of toxicity, such as the dioxin-like compounds in our example. Under an assumption of dose additivity, TEFs are used to predict the toxic effects of a mixture of chemicals based on knowledge about the potencies of its constituents. Relative to a reference, a chemical's TEF is a summary of available estimates of constant relative potency, even when similarity may not hold for all endpoints. One potential value of relative potency functions is to extend the TEF approach to better cope with chemicals having non-similar dose-response curves. Putzrath (1997) advocated expanding the TEF framework in a related way. This task will not be easy, however, as study- and endpoint-specific estimates of relative potency can vary by orders of magnitude (Walker *et al*, 2005), complicating broad summaries of potency.

In closing, researchers may draw incorrect conclusions about relative potency if their inferences are based on ED_{50} ratios alone or on dose-response curves forced to be similar when data suggest that they are not. Consequently, we recommend expressing relative potency as a function of dose, response, or response quantile and making inferences within this more general framework. Which relative potency function to use depends primarily on whether the response limits differ between chemicals and, if so, whether those differences are viewed as intrinsic or extrinsic to the comparison. Graphical displays of relative potency functions provide a useful visual summary; but, when appropriate, single-number or set-based summaries of the relative potency function may succinctly describe important aspects of relative potency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CI	confidence interval
ED₅₀	median effective dose
EROD	7-ethoxyresorufin- <i>O</i> -deethylase
NTP	National Toxicology Program
PCB126	3,3',4,4',5-pentachlorobiphenyl
PeCDF	2,3,4,7,8-pentachlorodibenzofuran
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TEF	toxic equivalency factor

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Appendix: Technical Details of Relative Potency Functions

Relative Potency as a Function of Mean Response μ

Using the notation in the manuscript, we denote the mean response to dose d_i of chemical C_i by $\mu_i = f(d_i; \theta_i)$ for $i = 0, 1$, where f is a strictly monotone dose-response model. Because μ_i is a monotone function of d_i , we can invert f and express d_i as a monotone function of μ_i . We denote the corresponding inverse function by $d_i = f^{-1}(\mu_i; \theta_i)$ for $i = 0, 1$.

For similar dose-response curves, as noted in the manuscript, whenever doses d_0 and d_1 satisfy $f(d_0; \theta_0) = f(d_1; \theta_1)$, the relative potency of C_1 compared with C_0 is $\rho = d_0 / d_1$. We extend this dose-ratio definition to non-similar dose-response curves by regarding the doses in the ratio as functions of mean response μ . Thus, dividing $d_0 = f^{-1}(\mu; \theta_0)$ by $d_1 = f^{-1}(\mu; \theta_1)$, we obtain relative potency as a function of μ :

$$\rho_\mu(\mu; \theta_0, \theta_1) = f^{-1}(\mu; \theta_0) / f^{-1}(\mu; \theta_1). \quad (\text{A.1})$$

Here, $\rho_{\mu}(\mu; \theta_0, \theta_1)$ is a relative potency function that varies with mean response μ and chemical-specific model parameters θ_0 and θ_1 ; for convenience, we use the abbreviated notation $\rho_{\mu}(\mu)$. Ritz *et al* (2006) proposed a formula equivalent to equation (A.1).

Let L_i and U_i be the lower and upper limits of mean response, respectively, for chemical C_i for $i = 0, 1$. Whenever the range of mean response for C_0 overlaps that for C_1 , namely, when $\max(L_0, L_1) < \mu < \min(U_0, U_1)$, $\rho_{\mu}(\mu)$ is positive and finite; whereas, for $\mu < \min(L_0, L_1)$ and $\mu > \max(U_0, U_1)$, $\rho_{\mu}(\mu)$ is undefined. When the lower (or upper) limits differ and the mean response μ lies between the two distinct lower (or upper) limits, the relative potency is 0 or ∞ , depending on which chemical has the more extreme limit. Graphically, when plotting the dose-response curves on a log-dose scale, if the horizontal line $\mu = r$ intersects both dose-response curves, the distance between the curves is finite and $\rho_{\mu}(r)$ is finite and positive. If the horizontal line intersects neither of the dose-response curves, $\rho_{\mu}(r)$ is undefined. If the horizontal line intersects only one of the dose-response curves, the distance between the curves can be regarded as being infinite. In this case, $\log(\rho_{\mu}(\mu)) = -\infty$ (i.e., $\rho_{\mu}(\mu) = 0$) if $U_0 > U_1$ or $L_0 > L_1$, and $\log(\rho_{\mu}(\mu)) = \infty$ (i.e., $\rho_{\mu}(\mu) = \infty$) if $U_0 < U_1$ or $L_0 < L_1$. Even when the two chemicals share the same response limits, $L_0 = L_1 = L$ and $U_0 = U_1 = U$, $\rho_{\mu}(\mu)$ sometimes takes one of the values 0 or ∞ for $\mu = L$ and the other value for $\mu = U$, depending on the values of other parameters in θ_0 and θ_1 (for example, if $S_0 \neq S_1$ in a Hill model).

Consider a Hill dose-response model. Setting the right-hand side of equation (1) in the main text equal to μ and solving for d in terms of μ gives a Hill-model-specific inverse function:

$$d = M[(\mu - L)/(U - \mu)]^{1/S} = f^{-1}(\mu; \theta). \quad (\text{A.2})$$

Substituting equation (A.2), suitably subscripted to distinguish test and reference chemicals, into equation (A.1) and simplifying yields the Hill-model-specific relative potency function:

$$\rho_{\mu}(\mu) = (M_0/M_1)[(\mu - L_0)/(U_0 - \mu)]^{1/S_0} / [(\mu - L_1)/(U_1 - \mu)]^{1/S_1}. \quad (\text{A.3})$$

Whenever C_0 and C_1 have the same lower and upper response limits, say $L_0 = L_1 = L$ and $U_0 = U_1 = U$, equation (A.3) reduces to:

$$\rho_{\mu}(\mu) = (M_0/M_1)[(\mu - L)/(U - \mu)]^{(1/S_0) - (1/S_1)}.$$

If $S_0 = S_1$ as well, then $\rho_{\mu}(\mu)$ is constant and equal to the ratio of ED_{50} s, namely, M_0/M_1 .

Relative Potency as a Function of Dose d_i

Because, with a monotone dose-response function, each value of μ corresponds to a unique value of d_I , we can re-express $\rho_{\mu}(\mu)$ as a function of d_I , thereby deriving relative potency as a function of d_I . Accordingly, we substitute $f(d_I; \theta_I)$ for μ in both the numerator and denominator of equation (A.1), and note that $f^{-1}(f(d_i; \theta_i); \theta_1) = d_1$, to obtain:

$$\rho_{d_1}(d_1; \theta_0, \theta_1) = f^{-1}(f(d_1; \theta_1); \theta_0) / d_1. \quad (\text{A.4})$$

Analogously for d_0 , we can substitute $f(d_0; \theta_0)$ for μ in equation (A.1) to obtain:

$$\rho_{d_0}(d_0; \theta_0, \theta_1) = d_0 / f^{-1}(f(d_0; \theta_0); \theta_1). \quad (\text{A.5})$$

Each $\rho_{d_i}(d_i; \theta_0; \theta_1)$ for $i = 0, 1$, or $\rho_{d_i}(d_i)$ for short, is a relative potency function that varies with d_i , θ_0 and θ_1 .

The values where each $\rho_{d_i}(d_i)$ is defined follow from those where $\rho_\mu(\mu)$ is defined. The function $\rho_{d_i}(d_i)$ is positive and finite for d_i values where the range of mean response for C_0 overlaps that for C_1 , namely, $\max(L_0, L_1) < f(d_i; \theta_i) < \min(U_0, U_1)$; $\rho_{d_i}(d_i)$ is undefined for d_i values corresponding to mean responses beyond the more extreme of the two limits; and otherwise $\rho_{d_i}(d_i)$ is zero or infinite.

Substituting explicit expressions for the Hill dose-response model from equation (1) and its inverse from equation (A.2), suitably subscripted to distinguish test and reference chemicals, into equations (A.4) and (A.5) and simplifying yields the following Hill-model-specific relative potency functions:

$$\rho_{d_1}(d_1) = \frac{M_0}{d_1} \left[\frac{(L_1 - L_0)(M_1^{S_1} + d_1^{S_1}) + (U_1 - L_1)d_1^{S_1}}{(U_0 - L_1)(M_1^{S_1} + d_1^{S_1}) + (U_1 - L_1)d_1^{S_1}} \right]^{1/S_0}, \quad (\text{A.6})$$

$$\rho_{d_0}(d_0) = \frac{d_0}{M_1} \left[\frac{(U_1 - L_0)(M_0^{S_0} + d_0^{S_0}) - (U_0 - L_0)d_0^{S_0}}{(L_0 - L_1)(M_0^{S_0} + d_0^{S_0}) + (U_0 - L_0)d_0^{S_0}} \right]^{1/S_1}. \quad (\text{A.7})$$

Whenever C_0 and C_1 have the same lower and upper response limits, equations (A.6) and (A.7), respectively, reduce to:

$$\rho_{d_1}(d_1) = (M_0/M_1) [d_1/M_1]^{(S_1/S_0)-1}, \quad (\text{A.8})$$

$$\rho_{d_0}(d_0) = (M_0/M_1) [M_0/d_0]^{(S_0/S_1)-1}. \quad (\text{A.9})$$

From equations (A.8) and (A.9), we see that $\rho_{d_i}(d_i)$ is a straight line when plotted with both axes on a log scale, that is, $\log(\rho_{d_i}(d_i)) = A_i + B_i \log(d_i)$ for some intercept A_i and slope B_i , both of which depend on parameters S_i and M_i , but not on L or U . For example, taking logs on both sides of equation (A.8) and rearranging terms shows that $\log(\rho_{d_i}(d_i))$ is linear in $\log(d_1)$:

$$\log(\rho_{d_1}(d_1)) = [\log(M_0) - (S_1/S_0)\log(M_1)] + [(S_1/S_0) - 1]\log(d_1).$$

The same argument applied to equation (A.9) shows that $\log(\rho_{d_0}(d_0))$ is linear in $\log(d_0)$. If, in addition, $S_0 = S_1$, then each $\rho_{d_i}(d_i)$ is constant and equal to the ratio of ED_{50} s, namely, M_0/M_1 .

Relative Potency as a Function of Response Quantile π

Finally, we can define relative potency as the ratio of $ED_{100\pi}$ s for C_0 and C_1 , where π is a response quantile as defined in the manuscript. Accordingly, we substitute $L_i + (U_i - L_i)\pi$ for μ_i in the formula $d_i = f^{-1}(\mu_i; \theta_i)$ and take the ratio d_0/d_1 to obtain:

$$\rho_{\pi}(\pi; \theta_0, \theta_1) = f^{-1}(L_0 + (U_0 - L_0)\pi; \theta_0) / f^{-1}(L_1 + (U_1 - L_1)\pi; \theta_1). \quad (\text{A.10})$$

Here, $\rho_{\pi}(\pi; \theta_0, \theta_1)$, or $\rho_{\pi}(\pi)$ for short, varies with θ_0 , θ_1 and response quantile π . From equation (A.10), we see that $\rho_{\pi}(\pi)$ is positive and finite for all $0 < \pi < 1$, though $\rho_{\pi}(\pi)$ can equal 0 or ∞ for values of π on the boundaries of the unit interval.

The function $\rho_{\pi}(\pi)$, defined as the ratio of $ED_{100\pi}$ s, embodies the usual concept of relative potency only when C_0 and C_1 have the same response limits. If $L_0 = L_1$ and $U_0 = U_1$, then $L_0 + (U_0 - L_0)\pi$ and $L_1 + (U_1 - L_1)\pi$ represent the same mean response μ , so the resulting concept of relative potency coincides with the usual concept. When response limits differ, $L_0 + (U_0 - L_0)\pi$ and $L_1 + (U_1 - L_1)\pi$ correspond to different values of μ , despite representing the same quantile of mean response, thereby modifying the usual concept of relative potency.

Despite the appearance of response limits L_i and U_i in equation (A.10), $\rho_{\pi}(\pi)$ does not depend on their values, as the following argument shows. A broad class of dose-response models for which the mean response is subject to lower and upper limits can be represented as:

$$f(d; \theta) = L + (U - L)g(d; \varphi), \quad (\text{A.11})$$

where $g(d; \varphi)$ is a monotone function of dose d with $0 < g(d; \varphi) < 1$, φ is a parameter vector, and $\theta = (L, U, \varphi)$. For a function that ranges from 0 to 1, multiplying by $(U - L)$ stretches the range so the upper limit is $(U - L)$; then adding L shifts both limits so the resulting range is from L to U . Thus, $f(d; \theta)$ is a stretched and shifted version of $g(d; \varphi)$. We represent the inverse of function $\mu^0 = g(d; \varphi)$ by the function $g^{-1}(\mu^0; \varphi) = d$ for $0 < \mu^0 < 1$. (It is important to distinguish μ and μ^0 because they have different ranges). Because d is the same in both functions, we have:

$$f^{-1}(\mu; \theta) = g^{-1}(\mu^0; \varphi). \quad (\text{A.12})$$

Substituting μ for $f(d; \theta)$ and μ^0 for $g(d; \varphi)$ in equation (A.11) yields $\mu = L + (U - L)\mu^0$, and then substituting this last expression into the left side of equation (A.12) yields:

$$f^{-1}(L + (U - L)\mu^0; \theta) = g^{-1}(\mu^0; \varphi). \quad (\text{A.13})$$

Now, μ^0 and π are equivalent because both variables range from 0 to 1. Thus, equation (A.13) may be rewritten by replacing μ^0 with π to get $f^{-1}(L + (U - L)\pi; \theta) = g^{-1}(\pi; \varphi)$. Substituting this expression into equation (A.10), with attention to the chemical-specific subscripts, yields:

$$\rho_{\pi}(\pi; \theta_0, \theta_1) = g^{-1}(\pi; \varphi_0) / g^{-1}(\pi; \varphi_1). \quad (\text{A.14})$$

Hence, equation (A.14) shows that $\rho_\pi(\pi)$ is free of response limits L_i and U_i ($i=0,1$) as claimed.

The preceding development also shows the relationship between $\rho_\pi(\pi)$ and the approach of ‘standardizing’ the response variables by shifting and rescaling them to have range 0 to 1. A relative potency function properly calculated from such standardized variables would be $\rho_\pi(\pi)$. Without standardization and for any dose-response model, the construction of $\rho_\pi(\pi)$, in effect, implicitly rescales the mean response of each chemical separately so that the corresponding dose-response curves have the same upper and lower limits.

Substituting the Hill-model-specific inverse formula of equation (A.2), with μ replaced by $L_i + (U_i - L_i)\pi$, suitably subscripted to distinguish test and reference chemicals, into equation (A.10) and simplifying yields the following Hill-model-specific relative potency function:

$$\rho_\pi(\pi) = (M_0/M_1) [\pi/(1-\pi)]^{(1/S_0)-(1/S_1)}. \quad (\text{A.15})$$

Equation (A.15) illustrates that, for a pair of Hill models, $\rho_\pi(\pi)$ does not depend on L_i and U_i and that $\rho_\pi(1/2) = M_0/M_1$ regardless of the other parameters. Moreover, the graph of $\rho_\pi(\pi)$ has either of two forms. If $S_0 \neq S_1$, then π drops out of equation (A.15) and $\rho_\pi(\pi)$ is constant and equal to M_0/M_1 whenever $0 < \pi < 1$. If $S_0 = S_1$, then $\rho_\pi(\pi)$ is a monotone (increasing or decreasing) function that crosses 1 exactly once, and $\log(\rho_\pi(\pi))$ is symmetric about $\log(M_0/M_1)$. To see this result, take logs on both sides of equation (A.15), which yields:

$$\log(\rho_\pi(\pi)) = \log(M_0/M_1) + [(1/S_0) - (1/S_1)] \log(\pi/(1-\pi)).$$

Define $h(\pi) = \log(\pi/(1-\pi))$ and note that $h(1/2) = 0$ and $h(\pi) = -h(1-\pi)$ for all $0 < \pi < 1$. For any $0 < \varepsilon < 1/2$, substituting $1/2 + \varepsilon$ for π in $h(\pi) = -h(1-\pi)$ gives $h(1/2 + \varepsilon) = -h(1/2 - \varepsilon)$. Thus, from the baseline value $\log(\rho_\pi(1/2)) = \log(M_0/M_1)$, the function $\log(\rho_\pi(\pi))$ increases (or decreases) by $[(1/S_0) - (1/S_1)] h(1/2 + \varepsilon)$ units for every increment (or decrement) of size ε .

Geometric Mean Relative Potency

The geometric mean of $\rho_\mu(\mu)$ over interval (a,b) , denoted $\overset{\sim}{\rho}_\mu^{(a,b)}$, is the anti-log of the mean of the log relative potency function. Let $\lambda(\mu) = \log(\rho_\mu(\mu))$ and define its mean over (a,b) as:

$$\bar{\lambda}_\mu^{(a,b)} = (b-a)^{-1} \int_a^b \lambda_\mu(x) dx. \quad (\text{A.16})$$

The geometric mean relative potency over the interval (a,b) is $\overset{\sim}{\rho}_\mu^{(a,b)} = \exp(\bar{\lambda}_\mu^{(a,b)})$. A geometric mean relative potency based on $\rho_{d_i}(d_i)$ or $\rho_\pi(\pi)$ can be defined in an analogous fashion.

Also, because $\log(\rho_\pi(\pi))$ is symmetric about $\log(M_0/M_1)$, we see from equation (A.16)

that $\bar{\lambda}_\pi^{(a,b)} = \log(M_0/M_1)$ and hence $\overset{\sim}{\rho}_\pi^{(a,b)} = M_0/M_1$ for any interval (a,b) centered at $\pi = 1/2$; that is, for any interval $(a,b) = (1/2 - \varepsilon, 1/2 + \varepsilon)$ with $0 < \varepsilon < 1/2$.

Figure 1. Similar dose-response curves. The reference and test chemicals are C_0 and C_1 , respectively. With dose plotted on a logarithmic scale, the horizontal distance between curves, denoted λ and equal to $\log(\rho)$, is constant. Each arrow extends from the C_1 curve to the C_0 curve at a different value of mean response; an arrow's length gives the magnitude and its direction (left being negative) gives the sign of λ (i.e., whether $\rho < 1$ or $\rho > 1$). Both dose-response curves have the same lower response limit (L), upper response limit (U), and shape, but different ED_{50} s as indicated by vertical lines at M_0 and M_1 for C_0 and C_1 , respectively.

Figure 2. Estimated dose-response curves for TCDD, PCB126, PeCDF and their TEF-based mixture. Panels: (a) separate Hill model fit for each chemical; and (b) Hill models constrained by assuming all four chemicals have similar dose-response functions. Curves: solid, TCDD; dotted, PCB126; dashed, PeCDF; and dot-dash, the mixture. Vertical lines indicate estimated ED_{50} s.

Figure 3.

Non-similar dose-response curves. The reference and test chemicals are C_0 and C_1 , respectively. With dose plotted on a logarithmic scale, the horizontal distance between curves is not constant. Panel (a) illustrates how the log relative potency's magnitude (arrow length) and sign (arrow direction) vary as functions of mean response, dose of C_0 , or dose of C_1 . Vertical lines in panel (a) indicate doses that correspond to specific mean responses. Panel (b) shows the correspondence with an alternative measure of log relative potency, which varies with response quantile π ; each diagonal line connects a pair of μ values that correspond to a specified value of π . All arrows in both panels begin at the same point along the curve for C_1 , but in panel (a) the arrows extend horizontally to the dose of C_0 that gives an identical response, whereas in panel (b) they represent the horizontal vector component that is the alternative measure of log relative potency.

Figure 4.

Examples of non-similar dose-response curves and corresponding relative potency functions. First column, labeled $f(d; \theta)$: dose-response curves for reference chemical C_0 (solid) and test chemical C_1 (dashed); vertical dotted lines indicate ED_{50} s. The parameter vector $\theta = (L, U, S, M)$ for C_0 is (20, 80, 3, 10) in every row; the parameter vectors for C_1 are (20, 80, 1, 100) in row (a), (5, 65, 3, 8) in row (b), and (10, 90, 1, 10) in row (c). Second column, labeled $\rho_\mu(\mu)$: relative potency as a function of mean response μ . Third column, labeled $\rho_d(d)$: relative potency as a function of dose d_i of C_i ; solid, $\rho_{d_0}(d_0)$, and dashed, $\rho_{d_1}(d_1)$. Fourth column, labeled $\rho_\pi(\pi)$: relative potency as a function of response quantile π . Horizontal lines: dotted, ratio of ED_{50} s (*i.e.*, $\rho = M_0/M_1$); and dot-dash, equipotency (*i.e.*, $\rho = 1$).

Figure 5.

EROD-activity dose-response data and estimated Hill-model dose-response curves for four chemicals. Panels: (a) TCDD, (b) PCB126, (c) PeCDF, and (d) their TEF-based mixture. Symbols: individual response (open circle), and dose-specific average response (solid triangle). Curves: solid, separate Hill model fit for each chemical; dashed, Hill models that enforce similar dose-response curves for all four chemicals. Vertical lines indicate estimated ED_{50} s.

Figure 6.

Estimated Hill-model dose-response curves for TCDD, PCB126, PeCDF, and their TEF-based mixture and corresponding relative potency functions (relative to TCDD). Hill models were fit separately for PeCDF and the mixture but were constrained by assuming similar dose-response curves for TCDD and PCB126. Panels: (a) $f(d; \theta)$, estimated dose-response curves; (b) $\rho_{\mu}(\mu)$, relative potency as a function of mean response; (c) $\rho_{d_0}(d_0)$, relative potency as a function of the dose of TCDD; and (d) $\rho_{\pi}(\pi)$, relative potency as a function of response quantile. Curves: solid, TCDD; dotted, PCB126; dashed, PeCDF; and dot-dash, the mixture. Vertical lines in panel (a) indicate estimated ED_{50} s.

Table 1

Summary of key features of relative potency functions.

<p>Non-constant relative potency can be expressed as a function of mean response (μ), reference chemical dose (d_0), test chemical dose (d_1), or response quantile (π); the corresponding relative potency functions are denoted $\rho_\mu(\mu)$, $\rho_{d_0}(d_0)$, $\rho_{d_1}(d_1)$, and $\rho_\pi(\pi)$, respectively.</p> <p>Each relative potency function is constructed from the dose-response curves for the two chemicals.</p> <p>If the dose-response curves for the reference and test chemicals are similar, all relative potency functions are constant functions with horizontal lines as graphs.</p> <p>If the reference and test chemicals have the same lower and the same upper limits of mean response, all four relative potency functions provide equivalent information, though their graphs look different because the x-axes differ.</p> <p>If the reference and test chemicals have different lower or different upper limits of mean response, relative potency functions $\rho_\mu(\mu)$, $\rho_{d_0}(d_0)$, $\rho_{d_1}(d_1)$ provide equivalent information but $\rho_\pi(\pi)$ is distinct and incorporates a modified definition of relative potency.</p> <p>If differences in response limits are extrinsic to the chemicals for the response under study, $\rho_\pi(\pi)$ is preferred because its construction ignores limit discrepancies.</p> <p>If differences in response limits are intrinsic to the chemicals for the response under study, relative potency functions $\rho_\mu(\mu)$, $\rho_{d_0}(d_0)$, $\rho_{d_1}(d_1)$ are preferred because their construction reflects limit discrepancies. Choice among these three depends on the application: $\rho_\mu(\mu)$ is convenient for ranking chemicals; $\rho_{d_0}(d_0)$, for converting from reference dose d_0 to test dose d_1; and $\rho_{d_1}(d_1)$, for converting from test dose d_1 to reference dose d_0.</p>

Table 2

Parameter estimates (standard errors) by chemical under unrestricted, partially similar, and fully similar Hill-model dose-response curves for the NTP liver enzyme (EROD) activity data.

Model	Parameter	Chemical			
		TCDD	PCB126	PeCDF	Mixture
Unrestricted ^a	<i>L</i>	29.9 (2.0)	39.8 (2.6)	77.2 (5.1)	70.9 (4.7)
	<i>U</i>	1863 (129)	2054 (84)	6960 (2581)	3458 (776)
	<i>S</i>	1.25 (0.21)	1.42 (0.13)	0.81 (0.09)	0.90 (0.49)
	<i>M</i>	6.0 (1.1)	34.9 (4.2)	207.6 (167.7)	10.9 (5.6)
Only TCDD and PCB126 Have Similar Curves ^b	<i>L</i>	— 34.5 (1.7) —	—	77.2 (5.3)	70.9 (4.8)
	<i>U</i>	— 1984 (73) —	—	6960 (2667)	3458 (802)
	<i>S</i>	— 1.34 (0.11) —	—	0.81 (0.09)	0.90 (0.51)
	<i>M</i>	6.5 (0.7)	34.5 (4.0)	207.6 (173.3)	10.9 (5.8)
All Four Chemicals Have Similar Curves ^c	<i>L</i>	—	— 50.5 (2.5) —	—	—
	<i>U</i>	—	— 2669 (169) —	—	—
	<i>S</i>	—	— 1.00 (0.08) —	—	—
	<i>M</i>	13.6 (2.6)	73.5 (15.0)	31.0 (6.0)	4.7 (1.6)

^a Individual Hill model for each chemical; i.e., *L* (lower response limit), *U* (upper response limit), *S* (shape), and *M* (median effective dose or ED50) are estimated separately for each chemical.

^b Hill models with *L*, *U*, and *S* constrained to be equal for TCDD and PCB126, while all other parameters are estimated separately.

^c Hill models with *L*, *U*, and *S* constrained to be equal across all chemicals, while *M* is estimated separately for each chemical.