# A Conceptual Model for Evaluating Relative Potency Data for Use in Ecological Risk Assessments

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## Introduction

For chemicals with a common mechanism of toxicity, relative potency factors (RPFs) allow dose and exposure measures to be normalized to an equivalent toxicity amount of a model chemical. In the case of AHR agonists, the model chemical is usually 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[1]. In 1997 the World Health Organization sponsored the development of consensus toxicity equivalence factors (TEFs) for mammals, birds, and fish[2]. Since the normalization to TCDD is toxicity based, the TEFs or alternative RPFs should be applied to either doses or concentrations that are consistent with the toxicity dose metric; normally concentrations in an organism's tissues or diet. In using the toxicity equivalence methodology, TEFs and/or RPFs serve as the bridge between exposure and effects characterizations for mixtures of PCDDs, PCDFs, and PCBs. In ecological risk assessments the large number of possible target species, variety of exposure scenarios, and differences in toxicity effects, both in laboratory studies and environmental settings, create a continuing need for sorting and selecting relative potency (ReP) data to obtain optimum TEF and/or RPF values for maximizing accuracy of site-specific toxicity equivalence concentration (TEC) calculations.

In the 1997 WHO process a tiered approach was followed in deriving the TEFs for fish and birds from the ReP data available. Effect endpoints were grouped into four tiers. Overt toxicity in developing embryos was given the most weight, followed in order by biochemical effects in developing embryos, biochemical effects in *in vitro* systems, and finally by QSAR relationships. The biochemical effect data were limited to CYP1A induction. In a 1998 workshop organized by U.S. EPA and U.S. DOI, international experts supported evaluation of ReP data for calculation of RPFs as alternatives to the TEFs when species and toxicity endpoint specificity are likely to improve the accuracy of a risk assessments[3]. Since then we have further evaluated these considerations and proposed a somewhat more comprehensive conceptual model for evaluating ReP data and selecting assessment-specific RPFs[4]. This developing conceptual model may also be useful for future revisions and additions to the WHO TEFs, as well as a framework for research planning and design.

## Materials and Methods

Development and refinement of the conceptual model for evaluating ReP data for use in ecological risk assessments is possible to the extent that relevant new effects data are reported and research advances provide new understanding of specific AHR mediated mechanisms of action associated with different toxicity pathways. One data gap is the magnitude of interspecies differences in RePs and the degree to which it is independent of interspecies differences in sensitivity to TCDD. Variability in RePs probably can be largely attributed to the interplay between toxicodynamic and toxicokinetic relationships that vary across species and endpoints. A final consideration for is diagnosis of the uncertainties associated with use of RPFs and TEFs in actual ecological risk assessments that allow comparison of population or individual responses to toxicity predictions using the toxicity equivalence approach.

## Results and Discussion

Data limitations inrisk assessments do not negate the need to consider uncertainties and make optimum choices for RPF or TEF values, consistent with the applicable mechanism of action and dose metric, for the particular species and effects of concern. The three dimensional matrix model in Figure 1 conceptualizes three essential categories of variables (degree of specificity for species, end points, and dose metrics) to consider when evaluating the applicability of ReP data associated with TEFs or RPFs and the types of uncertainty inherent to them. Using this concept, selection of TEFs or RPFs can be based on a three dimensional hierarchical approach involving use of the best available information relative to the ideal choice - a species-specific RPF for the endpoint of concern based on optimum dose metrics. Currently, the model's primary value is to allow a visualization of the complex factors that

influence the applicability of potentially diverse ReP data for specific risk assessment scenarios. This could include enhancement of efforts to describe uncertainties associated with RPF selections. Ultimately, the model may be helpful in describing research needs and developing more quantitative methods and guidance for selecting RPFs.

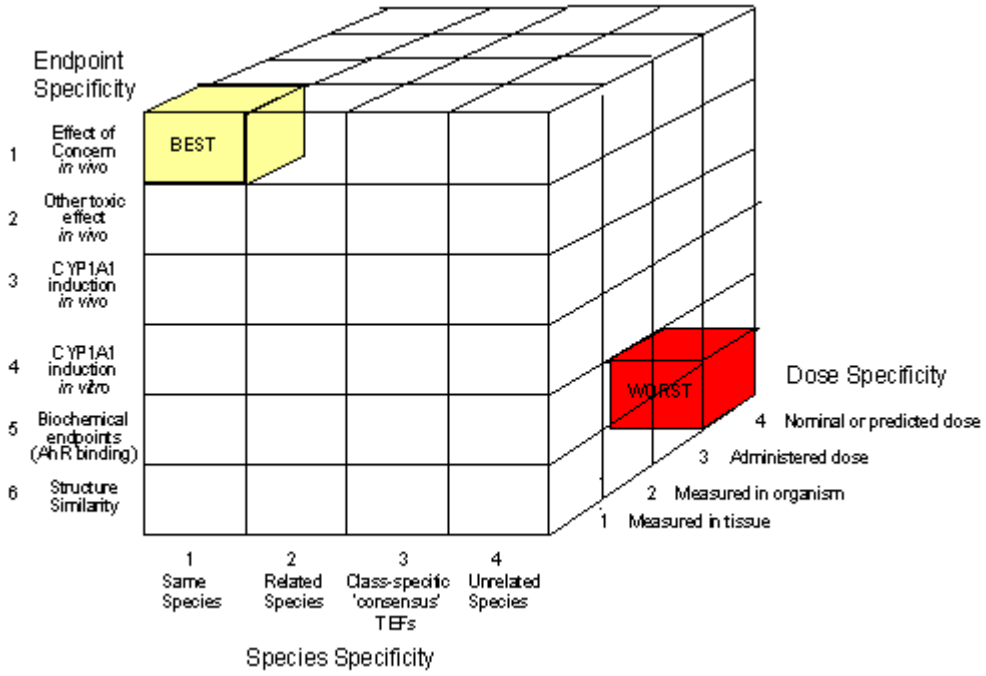


**Figure 1. Conceptual Model for selection of relative potency factors from available relative potency data. In ideal cases, uncertainty is minimized by use of ReP data from tier 1-1-1 (data in front upper left box).**

The issue of species- or endpoint-specific differences in RPFs is separate from that of species differences in sensitivity to TCDD which impacts the accuracy of the TCDD dose response relationship to be used. Limited ReP data for fish embryos (bull trout, lake trout, rainbow trout, and medaka) suggest that species sensitivity to TCDD is associated with smaller RPFs for PCB 126 when based on early life stage mortality. These differences in RPFs are less than proportional to the differences in species sensitivity. Two species that differ widely in their sensitivity to TCDD can have relatively similar RePs for most congeners. For example, chickens are 119-fold more sensitive than ducks for *in vitro* effects of TCDD, yet for TCDF and PCB congeners 126 and 81 the *in vitro*-based RPFs differ less than 5-fold between these species[5]. Similarly among fish, salmonids are the most sensitive species and zebrafish the least sensitive species to the early life stage toxicity caused by TCDD[6], yet RPFs based on zebrafish *in vitro* endpoints (i.e., CYP1A induction in liver) are generally within 5-fold of RePs determined in a variety of rainbow trout *in vitro* systems when the same endpoint in the same tissues are compared[7]. Analysis of rainbow trout and zebrafish RePs suggests that uncertainties surrounding application of the toxicity equivalence methodology are likely to be greater when applying TEFs or RPFs across tissues or endpoints than across fish species[7]. In summary, there are presently insufficient data to determine if there is a significant association between sensitivity to TCDD and RePs for different species.

The y axis of the conceptual model for selection of RPFs represents six tiers that correspond to the various *in vivo*, *in vitro*, and molecular similarity endpoints used currently to determine relative potency of congeners. The tiers represent a preferential ranking based on an assumption that prediction of organism mortality is the most likely application for RPFs in ecological risk assessments. The order of preference is similar to that used at the WHO workshop in deriving TEFs for fish, birds, and mammals[2]. The highest preference is given to RPFs determined for *in vivo* toxicity endpoints. Tier 1 is reserved for *in vivo* toxicity data for the endpoint of concern (e.g. early life stage mortality). Tier 2 is for other *in vivo* toxicity endpoints that may be less directly connected to the assessment endpoint of concern (e.g., growth or behavior). Tier 3 includes data for CYP1A1 induction *in vivo* and is followed

by CYP1A1 induction *in vitro* in Tier 4 because *in vitro* data tend to be less toxicokinetically realistic than *in vivo* data. Lower preference in Tier 5 is assigned to RPFs determined using biochemical endpoints, which are more distantly related to typical ecological assessment endpoints. A primary example of Tier 5 is AHR binding affinity which is very mechanistically connected to, but considerably upstream from, toxicities of concern. Consistent with the WHO TEF selection process[7], Tier 6 is reserved for chemical structure similarity approaches which may be more or less quantitative in comparing AHR agonist potencies to TCDD for a variety of endpoints.

The x axis in the matrix model for RPF selection indicates the phylogenetic relatedness of the species of concern to the species for which RPFs are to be applied. It is divided into four levels, reflecting different degrees of uncertainty, with uncertainty increasing from left to right. If ReP data are available for the species of concern (level 1 - same species), no interspecies extrapolation is involved in using these as RPFs, although other uncertainties such as endpoint extrapolation may still be an issue. If ReP data are available for a closely related species, a species within the same genus or family for example (level 2), uncertainty is greater due to potential species differences. The TEFs, although based in some cases on species-specific data, are based on class generalizations and are thus represented in the third level. In some cases TEFs may be based on a species closely related to the species of concern. In these cases the phylogenetic uncertainty is relatively less and the TEF may equate to one of the first two levels (same or related species). If ReP data associated with a TEF are from a more distantly related species within the same class, uncertainty increases (level 4). When level 4 data are in agreement with other ReP data for more related species (level 2), uncertainty is reduced for use of the level 4 data to determine an RPF for a specific chemical without level 2 ReP data.

The basis for the phylogenetic methodology reflected by the x axis of the three dimensional matrix model in Figure 1 for RPF selection is both theoretical and empirical. It assumes that two species that are more closely related phylogenetically will have RPFs (determined for the same endpoint) that are similar or identical. This methodology is supported by data such as that showing that the RPFs for PCB 126 to produce early life stage mortality in lake trout and rainbow trout vary by less than a factor of two[8]. However, it is clear that more data on the relative potency of congeners to produce various effects in additional species are necessary to more systematically test this assumption. Exceptions to this assumption for certain species or congeners may be revealed as additional data are collected. It is important to note that when RePs for different endpoints are compared, rank order potencies of AhR agonists appear to be conserved but RePs based on CYP1A1 induction tend to be greater than RePs based on early life stage mortality. For example, rainbow trout liver EROD, liver cell culture EROD, and gonad cell CYP1A1 mRNA assays all produce RePs that average six to ten times greater than RePs based on rainbow trout early life stage mortality[9]. This tendency for systematic differences related to organismal and biochemical response endpoints was considered in the WHO selection of TEFs for fish, birds, and mammals[2] and the TEF workshop recommendations for improving RPF selections[3].

The z axis of the conceptual model for RPF selection represents the degree to which the dose data associated with different sets of RePs are related to the effect of concern and the associated mechanism of action (specificity) and the TCDD dose-response relationship chosen for the assessment (consistency). To the extent dose specificity is related to the endpoint and species associated with each candidate set of RPFs, it may be best considered after characterizing the endpoint and species specificity of available RePs. A universal concern is the specificity and accuracy of the analytical methodology used for the available ReP data. It is more difficult to regard evaluation of dose specificity and consistency as a simple tiered process. Because of the complexity of dose metric impacts on RPF choices, evaluation of potential systematic errors associated with the analytical methodology should probably be accomplished as a final step in choosing RPFs.

Concentrations of chemicals measured in specific tissues of organisms or cell cultures, at a time most closely reflecting potency for causing the effect, are optimum expressions for doses associated with AHR mediated toxicity and can be placed in dose specificity Tier 1, if this is consistent with the TCDD dose-response relationship chosen for the assessment. RPFs based on measured concentrations in fish embryos close to fertilization in association with subsequent mortality are good examples of Tier 1. RPFs based on *in vivo* CYP1A1 induction in fish would also fall into Tier 1 if concentrations of chemicals are measured at the appropriate time in the appropriate tissues. Dose specificity Tier 2 incorporates uncertainties and systematic differences affecting measurements of administered doses (typically external to the organism or cell culture) associated with changes in concentrations during chemical uptake and distribution through different routes of exposure. An example is the effect metabolism in the organism may have

on the relative amounts of TCDD and test chemical *in vivo* in comparison to the relative amounts in the administered doses (e.g. in diet, water, sediment/soil, injection). As with Tier 1, Tier 2 assumes that the dose is consistent with the TCDD dose-response relationship chosen for the assessment. Dose specificity Tier 3 includes nominal (not based on measurement of concentrations in exposures) or predicted (based on mechanisms of fate and uptake during exposures) doses. In other words, Tier 3 includes both estimated/predicted *in vivo* doses and administered doses which are not determined by direct measurement during the test. Most *in vitro* effects based ReP data probably fall in Tier 3 of this axis, rather than Tier 2, because concentrations of the chemicals are often not measured in the cell cultures.

The consequences of inconsistencies between dose metrics used for RePs and the dose metrics involved with the TCDD dose-response relationship chosen for an assessment are varied but should be considered. Dose specificity Tier 4 includes ReP data that would be in Tiers 1 or 2, if such inconsistencies were not present. A hypothetical example might be use of a largemouth bass early life stage mortality response relationship based on concentration of TCDD in food of females during ovulation. The selection of the fish TEFs which have dose as concentrations measured in rainbow trout eggs would create a dose inconsistency associated with Tier 4. This inconsistency could be avoided and Tier 1 dose specificity/consistency achieved if the concentrations of TCDD associated with largemouth bass early life stage mortality were measured in the largemouth bass eggs. Inconsistencies involving application of RPFs based on administered doses to TCDD dose-response relationships based on measured dose *in vivo* would also be associated with Tier 4. Dose data suspected of having significant errors that increase uncertainty for the use of an associated ReP as an RPF, effectively place the RPF in a lower dose specificity tier. An example of data which could fall into this category is the presence of more potent impurities in test chemicals that could cause the observed effects. For example, toxic PCDFs have been found as contaminants of some PCB congener standards[10, 3]. Contamination of test samples usually becomes a problem when the contaminant causes the relative potency of the test chemical to be overestimated. Other sources of dose measurement errors may be related to limitations of analytical methods.

**Conclusions**

We have found the conceptual model useful for diagnosing and describing uncertainties associated with selection and use of TEFs and RPFs in ecological risk assessments. The model supports sensitivity analyses using alternative RPF values. Use of ReP data in risk assessments can vary from very specific, as in the case of lake trout in Lake Ontario[11], to complex extrapolations across species, end points, and dose metrics. The model can become more sophisticated as research fills some of the data gaps associated with the greatest sources of uncertainty.

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