A Toxicological Basis to Derive a Generic Interspecies Uncertainty Factor

Edward J. Calabrese and Linda A. Baldwin
School of Public Health, University of Massachusetts, Amherst, MA 01003 USA

The use of an uncertainty factor (UF) to account for interspecies variation in risk assessment procedures for noncarcinogens is well known and implemented by regulatory agencies at the federal and state levels. The approach that has been widely adopted is to assume that humans may be 10-fold more sensitive than the animal model. This factor of 10 has become routinely adopted in essentially all risk assessment procedures involving animal model data for extrapolation.

Despite the long-standing use of the interspecies UF of 10, only limited biological and/or toxicological justification for the interspecies UF has ever been put forth by any regulatory agency (1) or national advisory committee (e.g., National Academy of Sciences Safe Drinking Committee). The adoption of the 10-fold factor appears to have been based on a combination of public health protection philosophy, practical/intuitive toxicological insights based on experience, and a sense that it achieves its goal of protecting human health. The present paper offers what the authors believe to be a toxicological and statistically defensible foundation for deriving the interspecies UF, its database requirements, and statistical procedures for its derivation. In brief, the recommended interspecies UF is defined as the 95% of the population of 95% prediction intervals (PI) for binary interspecies comparisons based on phylogenetic relatedness. More specifically, the UF is derived by determining the minimum ratio of the estimated toxicity value and its 95% upper or lower PI after back-transformation from the logarithmic expression.

This paper presents the toxicological and statistical basis for this proposal and its implications for judging the reliability of current regulatory interspecies UF procedures as well as offering a fundamentally novel approach to deriving an interspecies UF.

An extensive database on interspecies variation in susceptibility to toxic agents exists in the aquatic toxicology area. The toxicity data are principally, though not exclusively, based on acutely toxic responses. The data are arranged in the form of binary interspecies comparisons with respect to toxicity from dozens to over 500 agents depending on the specific binary comparison. A binary comparison in the present context involves comparing the responses of two species to agents that were tested in both species. For example, two species of fish (e.g., smallmouth bass and perch) have been used to test over 500 of the same toxicants (Fig. 1). A binary comparison of these two species would include more than 500 agents. These data have been organized to assess whether a mathematical relationship exists such that the LC50 of one species may be a useful predictor of the LC50 in the other species via the use of regression modeling.

The above binary comparison methodology has been used by various authors (2-4) to estimate the LC50 for any new chemical in an untested species (e.g., smallmouth bass) if the LC50 were known for the perch. The estimate is made by calculating a prediction interval (PI) for the unknown chemical. Barnthouse et al. (5) have provided 95% PI estimates for numerous binary interspecies comparisons and organized them via phylogenetic relatedness. For example, interspecies comparisons were provided when the comparisons represented species-within-genus, genera-within-family, families-within-order, and orders-within-class comparisons. For example, in Figure 2 a species-within-genus comparison would represent a binary comparison of species 1 with species 2. A genera-within-family binary comparison would be represented by a comparison of species 1 with species 3. The reason for organizing the comparisons in this phylogenetic manner is the assumption that interspecies variation in susceptibility would increase as the phylogenetic distance increased.

Table 1 provides a summary of the database of phylogenetically based interspecies binary comparisons. The 95% PI for each binary comparison is provided, along with the number of different chemical agents tested for each binary comparison. The weighted mean value indicates that in general the closer the animal species were related, the smaller the 95% PI. The range of weighted means of 95% PI is from a low of 6.0 (species within genus) to a high of 26.0 for the orders-within-class grouping.

Slooff et al. (4) transformed the concept of the 95% PI into a 95% UF. Figure

Figure 1. Natural logarithms of LC50 values for Perciformes plotted against Salmoniformes (orders of the same class, Osteichthyes). The solid line represents the least-squares linear regression of the natural logarithm of LC50 values for Perciformes species on the natural logarithm of LC50 values for Salmoniformes species. Each circle represents the LC50 value of a specific chemical for both species. The number of chemicals represented in the figure is 505. Data from Johnson and Finley (8).

Address correspondence to E. J. Calabrese.
Received 21 June 1993; accepted 2 October 1993.
3 presents a graphic foundation of the PI as well as statistical definition and relationship to the UF concept. Thus, the species-within-genus 95% UF, as anticipated, is considerably smaller than the 95% UF for orders within class. The magnitude of interspecies variation in 95% PI values follows fairly closely with phylogenetic relatedness, as expected. Inconsistencies such as the similar estimates for species within genus and genera within family are likely related to issues concerning representativeness, number of binary comparisons, and number and nature of chemical agents tested.

The binary comparison values do not represent the population (or universe) of such values but must be considered a sample of the population. No knowledge exists concerning how representative this sample of values would be of the population. For the sake of argument, the samples of each phylogenetic subgroup are considered representative of their respective population values. Table 2 provides an estimate of upper 95% (using logistic regression modeling) of the population of 95% PI values (see Figure 3 for derivation of 95% PI values) according to phylogenetic relatedness. The unexpectedly high value from the families-within-order extrapolation group is partially inconsistent with the proposed phylogenetic relationship. This inconsistency is principally a result of the low number of binary comparisons \((N=7)\) and high variability of individual estimates in the families-within-order comparison group. This value is less stable than the orders-within-class grouping. Given the amount of data, the orders-within-class comparison offers the most stable and reliable perspective. We propose that these values can be used to provide a toxicologically and statistically based foundation for generic interspecies UF values when normalized for phylogenetic relatedness. The data suggest that four different UF values can be used, according to phylogenetic relatedness. The choice of 95% UF values would range from a low of 10 for the species within genus to a high of 65 for the orders within class. The genera-within-family and families-within-order groupings are more difficult to determine. Based on the phylogenetic relatedness concept, these two groups are estimated to be intermediary between the boundary values (i.e., species within genus, orders within class), approximating 25 and 50.

The proposed methodology approach takes into account two critical components in any interspecies UF estimation process: the need to address the universe of species (as is done via the use of logistic regression) and the need to incorporate the new chemicals (as is accomplished via the use of the PI approach). These findings and interpretations are based directly on data derived from

![Figure 2](image.jpg)

**Figure 2.** Interspecies comparisons based on phylogenetic relatedness. \(S_i\) represents a species for which data are available. \(S_i\) and \(S_j\) represent a species-within-genus comparison; \(S_i\) and \(S_j\) represent a species-within-family comparison; \(S_i\) and \(S_j\) represent a families-within-order comparison; and \(S_i\) and \(S_j\) represent an orders-within-class comparison.

**Table 1.** Taxonomic extrapolation: means and weighted means calculated for the 95% and 99% prediction intervals (PI) for uncertainty factors calculated from regression models (2)

| X variable | Y variable | n | Uncertainty factor |
|------------|------------|---|--------------------|
| **Taxonomic extrapolation: species within genera** | | | |
| *Salmo clarkii* | *S. gairdneri* | 18 | 9 | 13 |
| *S. clarkii* | *S. salar* | 6 | 6 | 10 |
| *S. gairdneri* | *S. trutta* | 8 | 6 | 8 |
| *Salmo salar* | *S. trutta* | 10 | 7 | 11 |
| *Lepomis mekels* | *I. punctatus* | 12 | 5 | 7 |
| *Lepomis cyanellus* | *L. macrocharis* | 14 | 6 | 9 |
| *Fundulus heteroclitus* | *F. majalis* | 12 | 6 | 8 |
| **Mean** | | 6.1 | 10.1 |
| **Weighted mean** | | 6.0 | 7.4 |
| **Taxonomic extrapolation: genera within families** | | | |
| *Oncorhynchus* | *Salmo* | 56 | 5 | 6 |
| *Oncorhynchus* | *Salvelinus* | 13 | 4 | 5 |
| *Salmo* | *Salvelinus* | 56 | 5 | 7 |
| *Carassius* | *Cyprinus* | 8 | 4 | 6 |
| *Carassius* | *Pimephales* | 19 | 7 | 9 |
| *Cyprinus* | *Pimephales* | 10 | 7 | 10 |
| *Lepomis* | *Micropterus* | 30 | 8 | 11 |
| *Lepomis* | *Pomoxis* | 8 | 9 | 13 |
| *Cyprinodon* | *Fundulus* | 12 | 6 | 8 |
| **Mean** | | 6.1 | 8.3 |
| **Weighted mean** | | 5.8 | 7.7 |
| **Taxonomic extrapolation: families within orders** | | | |
| *Centrarchidae* | *Percidae* | 47 | 10 | 14 |
| *Centrarchidae* | *Cichlidae* | 6 | 4 | 6 |
| *Percidae* | *Cichlidae* | 5 | 13 | 24 |
| *Salmonidae* | *Esocidae* | 11 | 9 | 13 |
| *Atherinidae* | *Cyprinodontidae* | 32 | 7 | 9 |
| *Mugilidae* | *Labridae* | 55 | 55 | 78 |
| *Cyprinodontidae* | *Poeclidae* | 12 | 3 | 5 |
| **Mean** | | 14.4 | 21.3 |
| **Weighted mean** | | 12.6 | 17.9 |
| **Taxonomic extrapolation: orders within classes** | | | |
| *Salmoniformes* | *Cypriniformes* | 225 | 20 | 27 |
| *Salmoniformes* | *Siluriformes* | 203 | 39 | 51 |
| *Salmoniformes* | *Perciformes* | 443 | 12 | 16 |
| *Cypriniformes* | *Siluriformes* | 111 | 11 | 15 |
| *Cypriniformes* | *Perciformes* | 219 | 32 | 43 |
| *Siluriformes* | *Perciformes* | 190 | 63 | 83 |
| *Anguilliformes* | *Tetradontiformes* | 12 | 13 | 18 |
| *Anguilliformes* | *Perciformes* | 34 | 25 | 34 |
| *Anguilliformes* | *Gasterosteiformes* | 8 | 16 | 24 |
| *Anguilliformes* | *Atheriniformes* | 48 | 9 | 12 |
| *Atheriniformes* | *Cypriniformes* | 7 | 501* | 786* |
| *Atheriniformes* | *Tetradontiformes* | 48 | 13 | 17 |
| *Atheriniformes* | *Perciformes* | 148 | 25 | 33 |
| *Atheriniformes* | *Gasterosteiformes* | 36 | 20 | 27 |
| *Gasterosteiformes* | *Tetradontiformes* | 8 | 20 | 30 |
| *Gasterosteiformes* | *Perciformes* | 33 | 32 | 43 |
| *Perciformes* | *Tetradontiformes* | 34 | 25 | 34 |
| **Mean** | | 23.5 | 31.7 |
| **Weighted mean** | | 26.0 | 34.5 |

*Not included in calculations.
acute toxicity experiments in fish. It assumes that the concept of phylogenetic relatedness in relationship to toxicity that is seen within fish species would apply to mammals and that the magnitude of the phylogenetic differences observed among fish species would be quantitatively comparable to mammalian toxicology.

The proposed methodology offers a number of important strengths in providing a foundation for the interspecies UF deriva-
tion: 1) it represents an extensive database obtained via a standardized testing protocol with respect to a critical integrative endpoint (i.e., LC₅₀); 2) it has the capability to incorporate phylogenetic relatedness to the predictive endpoint, which represents a significant advance and is entirely consistent with the biologically persuasive evolutionary paradigm of modern molecular biology relating genetic factors to susceptibility and/or resistance to chemical insults; 3) the database considers a large number of species representing different sizes, various biological adaptations, and variation in susceptibilities; 4) the database is composed of assessments of more than 400 different chemical agents representing several dozen chemical classes (e.g., pesticides, metals, PAHs, etc.). The database has the capacity to provide strong generalizations to account for both inherent species variation and large numbers of chemical agents; 5) the database permits the application of statistical evaluation to describe the distribution of responses with respect to both PIs for specific chemical responses and species variation in responses.

An area of potential concern with the present proposal is that the database is drawn entirely from aquatic models and is being generalized to mammalian phylogeny. The issue is not whether fish are effective qualitative/quantitative predictors of mammalian/human responses. Rather, the issue is whether the variation in response among species at the various levels of phylogenetic relatedness for the aquatic models is predictive of the mammalian phylogenetic variability that would be seen among mammalian models and humans for the same chemical contaminants. On a conceptual level, the trend in increased variability in susceptibility as seen in fish as the phylogenetic relatedness decreases would be expected to occur with mammalian systems. How quantitatively similar the weighted mean 95% PIs of the fish comparisons would be for the various phylogenetic relatedness comparisons in mammals is unknown. However, the use of biological systematics to provide a common measure of evolutionary/biological relatedness among the various animal classifications (e.g., fish and mammals) is a valuable and powerful tool that has rarely been applied to the field of toxicology/risk assessment. For example, the basic unit of comparison, the species, is similarly defined in fish as well as mammals. Although less precise than the species concept, the same conceptual definitions proceeds to broader categories (genus to class) across the animal kingdom. Thus, the trend of interspecies variability observed in various phylogenetic related categories in fish would be expected to be qualitatively similar in mammals as well.

Another area of possible concern is that the database uses acute rather than chronic toxic responses. This does not appear to be a serious concern because acute responses have been shown to be effective predictors of chronic effects of both a carcinogenic (5) and noncarcinogenic nature (3,4,6,7). In fact, the chronic no-observed adverse effect level in mammalian models and the chronic maximum acceptable toxicant concentration in fish have been similarly estimated by dividing the acutely lethal dose (LD₅₀/100) by approximately 50–75 (4,6,7,8). These data show a high degree of fundamental concordance between fish and mammalian responses with respect to the capacity of acute doses to estimate chronic responses.

There is a need to define the biological and statistical meaning of the interspecies
UF. The 95% UF as described here represents the upper 95% of the distribution of binary interspecies comparison 95% PI values. This is interpreted as 95% of experiments in which a chemical is tested would respond within the given PI (i.e., 95% PI). This also is interpreted to mean that 95% of every 100 unknown chemicals tested would display a response within the calculated PI. The 95% PI can, therefore, be used as a measure of interspecies variation. We then estimate the upper 95% of these "individual measures of interspecies variation" (i.e., the distribution of the 95% PI). This is then collectively interpreted as the following: 95% of chemicals would not exceed a given PI in 95% of species tested. The risk assessor has the flexibility to change the size of the PI as well as that portion of the logistic distribution deemed suitable for UF selection. For example, if the 99th percentile of the population of 95% PI were selected for the UF, then the range of phylogenetic UF values would be increased from the 10- to 65-fold range to the 16- to 87-fold range (Table 2). The final selection of which range of UF values to select would be based on value judgments.

The field of mammalian toxicology in which mice, rats, gerbils, guinea pigs, cats, and dogs are used as models to estimate human responses represents orders-within-class comparisons. Using the scheme outlined above suggests that the UF for such comparisons could range from 65 to 87 (possibly rounded to 50–100) rather than the 10-fold value currently used, depending on which quantitative estimate for UF derivation were selected.

REFERENCES

1. Dourson ML, Stara JF. Regulatory history and experimental support of uncertainty (safety) factors. Regul Toxicol Pharmacol 3:224–238 (1983).
2. Suter GW II, Vaughan DS, Gardner RH. Risk assessment by analysis of extrapolation error, a demonstration for effects of pollutants on fish. Environ Toxicol Chem 2:369–378 (1983).
3. Barnthouse LW, Suter GW, Rosen AE. Risks of toxic contaminants to exploited fish populations: influence of life history, data uncertainty and exploitation intensity. Environ Toxicol Chem 9:297–311 (1990).
4. Slooff W, Van Oers JA, DeZwart D. Margins of uncertainty in ecotoxicological hazard assessment. Environ Toxicol Chem 5:841–852 (1986).
5. Ziel E, Crouch EAC, Wilson R. A possible relationship between toxicity and carcinogenicity. J Am Coll Toxicol 5(2):137–151 (1986).
6. Layton DW, Mallon BJ, Rosenblatt DH, Small MJ. Deriving allowable daily intakes for systemic toxicants lacking chronic toxicity data. Regul Toxicol Pharmacol 7:96–112 (1987).
7. Kenaga EE. Predictability of chronic toxicity from acute toxicity of chemicals in fish and aquatic invertebrates. Environ Toxicol Chem 1:347–358 (1982).
8. Calabrese EJ, Baldwin L. Performing ecological risk assessments. Chelsea, MI: Lewis Publishers, 1993.
9. Johnson WW, Finley MT. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. Washington, DC: U.S. Fish and Wildlife Service Resource Publication 137. U.S. Department of the Interior, 1980.
10. Van Straalen NM, Denneman CAJ. Ecotoxicological evaluation of soil quality criteria. Ecotoxicol Environ Safety 18:241–251 (1989).