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Web-based Interspecies Correlation Estimation (Web-ICE) for Acute Toxicity: User Manual

Version 3.1

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Reference Web-ICE as:

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Web-ICE models may vary among versions as model data are updated and quality criteria refined. Please refer to the user manual available with each version for database descriptions.

Erratum

Page 5: The database of acute toxicity used in development of aquatic ICE models included 5487 EC/LC50 values of 180 species and 1258 chemicals.

Page 6: The models within Web-ICE are considered type II regressions based on the errors in variable, but were parameterized using the methods of type I based on Sokal and Rohlf (1995).

Contents

Abstract

Predictive toxicological models are integral to ecological risk assessment because data for most species are limited. Web-based Interspecies Correlation Estimation (Web-ICE) models are least square regressions that predict acute toxicity (LC50/LD50) of a chemical to a species, genus, or family based on estimates of relative sensitivity between the taxon of interest and that of a surrogate species. Web-ICE 3.0 includes a total 1440 models for aquatic taxa and 852 models for wildlife taxa. For aquatic species within the same family, Web-ICE models predict within 5-fold and 10-fold of the actual value with 91 and 96% certainty, respectively. For two species within the same order, aquatic models predict within 5-fold and 10-fold of the actual value with 86 and 96% certainty, respectively. Overall for wildlife species, Web-ICE predicts toxicity within 5-fold of the actual value with 85% certainty and within 10-fold of the actual value with 95% certainty. Models predict within 5-fold and 10-fold of the actual value with 90 and 97% certainty for wildlife surrogate and predicted taxa within the same order. For both aquatic and wildlife taxa, model certainty increases with decreasing taxonomic distance. Web-ICE 3.0 improves on earlier versions with the inclusion of an endangered species module, improved functionality of the SSD module, and more rigorous standardization of toxicity data.

Introduction

Information on the acute toxicity to multiple species is needed for the assessment of the risks to, and the protection of, individuals, populations, and ecological communities. However, toxicity data are limited for the majority of species, while standard test species are generally data rich. To address data gaps in species sensitivity, the Interspecies Correlation Estimations (ICE) application was developed by the U.S. Environmental Protection Agency (US EPA) and collaborators to extrapolate acute toxicity to taxa with little or no acute toxicity data, including threatened and endangered species (Asfaw et al. 2003). Web-based Interspecies Correlation Estimations (Web-ICE) provides interspecies extrapolation models for acute toxicity in a user-friendly internet platform.

ICE models estimate the acute toxicity (LC50/LD50) of a chemical to a species, genus, or family with no test data (the predicted taxon) from the known toxicity of the chemical to a species with test data (the surrogate species). ICE models are least square regressions of the relationship between surrogate and predicted taxon based on a database of acute toxicity values: median lethal water concentrations for aquatic species (LC50; µg/L) and median lethal oral doses for wildlife species (LD50; mg/kg bodyweight). ICE models can be used to estimate acute toxicity when a toxicity is known for a surrogate species or it can be estimated (e.g., QSAR), and there is an existing ICE model between the surrogate and taxa of interest (e.g., species-species; species-genus; species-family).

In addition to direct toxicity estimation from a surrogate species to predicted taxa, Web-ICE contains a Species Sensitivity Distribution (SSD) module that estimates the toxicity of all predicted species available for a common surrogate. Acute toxicity values generated by Web-ICE are expressed as a logistic cumulative probability distribution function in the SSD module to estimate an associated Hazardous Concentration (HC) or Hazardous Dose (HD) (Dyer et al. 2006). For example, the HC5 corresponds to the 5th percentile of the log-logistic species sensitivity distribution and is assumed to be protective of 95% of tested species. ICE-generated SSD hazard levels have been shown to be within an order of magnitude of measured HC5s (Dyer et al. 2006, Dyer et al. 2008) and HD5s (Awkerman et al. 2008) and provide additional information for ecological risk assessment.

This manual provides step-by-step instructions for using Web-ICE, as well as information on the expanded databases, model development, model validation, and user guidance on model selection and interpretation. User guidelines outlined in the *Guidance for Model Selection and Use* section of this manual should be followed to ensure high confidence and low uncertainty in model predictions used in risk assessment. Web-ICE 3.0 improves on earlier versions with the inclusion of an endangered species module, improved functionality of the SSD module, and more rigorous standardization of toxicity data.

Methods

I. Database Development

Aquatic (Fish and Invertebrates)

The database of acute toxicity used in development of ICE models included 5501 EC/LC50 values of 180 species and 1266 chemicals. The database was compiled from the following EPA[1] and public domain sources:

- US EPA ECOTOX (http://cfpub.epa.gov/ecotox/; accessed February 2009)
- US EPA Office of Pesticide Programs ecotoxicity database (accessed January 2007)
- US EPA Office of Water Ambient Water Quality Criteria (US EPA 1986)
- US EPA OPPT PreManufacture Notification (PMN)
- US EPA OPPT High Production Volume (HPV) Challenge Program
- US EPA Office of Research and Development data sources
- Mayer and Ellersieck 1986
- Open literature (for list of references, see Raimondo et al. 2008, 2009)

Data used in model development adhered to standard acute toxicity test condition requirements of the American Society for Testing and Materials (ASTM 2007, and earlier editions) and the US EPA Office of Prevention, Pesticides, and Toxic Substances (US EPA 1996). Data were standardized for test conditions and organism life stage to reduce variability (Appendix I). In short, selection criteria for aquatic test data were as follows:

- Reported chemical name or structure with chemical active ingredient $\geq$ 90%
- Open-ended toxicity values (i.e. > 100 mg/kg or <100 mg/kg) were excluded
- Endpoint was death (LC50) or immobilization (EC50)
- 48h EC/LC50 for daphnids, midges and mosquitoes; 96h EC/LC50 for fish and all other invertebrates
- Juvenile only for fish, amphibians, insects, molluscs, decapods; all life stages for other groups (Raimondo et al. 2009)
- Water quality parameters reported for test condition (e.g., temperature, salinity) or confirmation that test conditions met appropriate guideline conditions (e.g., GLP, previously reviewed OPP ecotoxicity data)
- Water quality parameters provided for normalization of metals, ammonia and pentachlorophenol as directed by Ambient Water Quality Criteria (e.g., AWQC; US EPA 1986)

[1] All confidential business information (CBI) and data have been censored.

When there was more than one toxicity value reported from multiple sources for a species and chemical, the geometric mean of the values were used. In cases where the range of minimum and maximum values for a chemical and species were greater than 10-fold, all data records for that chemical were removed for that species due to their high variability. Toxicity test values for specific compounds were normalized according to Ambient Water Quality Criteria procedures (e.g., specific metals adjusted to 50 mg/L hardness; reported on element basis; pentachlorophenol and ammonia were temperature and pH normalized; US EPA 1986). The resulting aquatic database was used to develop models to predict toxicity to a species, genus, or family from a surrogate species (see Appendix II).

Wildlife (Birds and Mammals)

The wildlife database was comprised of 4329 acute, single oral dose LD50 values (mg/kg body weight) for 156 species and 951 chemicals. The data were collected from the open literature (Hudson et al. 1984; Shafer and Bowles 1985, 2004; Shafer et al. 1983; Smith 1987) and from datasets compiled by governmental agencies of the United States (US EPA) and Canada (Environment Canada) (Baril et al. 1994; Mineau et al. 2001). Data were standardized by using only data for adult animals and data for chemicals of technical grade or formulations with $\geq$ 90% active ingredient. Open-ended toxicity values (i.e. > 100 mg/kg or <100 mg/kg) and duplicate records among multiple sources were not included in model development. When data were reported as a range (ie. 100-200 mg/kg; Hudson et al. 1984) or data were collected from multiple sources for a species and chemical, the geometric mean of the values was used. In cases where the range of minimum and maximum values for a chemical and species were greater than 10-fold, all data records for that chemical were removed for that species due to their high variability. Models derived from this wildlife database may be used to predict toxicity to a species or family from a surrogate species. Genus level models were not developed from the wildlife database because there were limited genera that had two or more species (See Appendix III), which is a requirement for development of higher taxa models.

II. Model Development

Models were developed using least squares methodology in which both variables are independent and subject to measurement error (Asfaw et al. 2003). For species-level models developed from aquatic and wildlife databases, an algorithm was written in S-plus (Insightful 2001) to pair every species with every other species by common chemical. Three or more common chemicals per pair were required for inclusion in the analysis. For each species pair, a linear model was used to calculate the regression equation $\text{Log}_{10}(\text{predicted toxicity}) = a + b*\text{Log}_{10}(\text{surrogate toxicity})$, where *a* and *b* are the intercept and slope of the line, respectively. Genus (aquatic only) and family-level models were similarly developed by pairing each surrogate species with each genus or family by common chemical. Predicted genera and families required unique toxicity values for two or more species within the taxon. Toxicity values for the surrogate species were removed in cases where it was compared to its own genus or family. ICE models were only developed between two aquatic taxa or two wildlife taxa; there are no models to predict toxicity to aquatic taxa from a wildlife species, or vice versa.

Only models that had a significant relationship (p-value $\leq$ 0.05) are included in Web-ICE. The following summarizes the number of significant models developed from the aquatic and wildlife databases for different taxonomic levels:

1) Aquatic species: 780 models comparing 77 species to 77 species;
2) Aquatic genera: 289 models comparing 62 species to 28 genera;
3) Aquatic family: 374 models comparing 69 species to 27 families;
4) Wildlife species: 560 models comparing 49 species to 49 species;
5) Wildlife family: 292 models comparing 49 species to 16 families.

III. Model Validation

The uncertainty of each model was assessed using leave-one-out cross-validation (Insightful 2001). In this method, each pair of acute toxicity values for surrogate and predicted taxa were systematically removed from the original model. The remaining data were used to rebuild a model and estimate the toxicity value of the removed predicted taxa toxicity value from the respective surrogate species toxicity value. This method could only be used for models with degrees of freedom equal to or greater than 2 (N $\geq$ 4). To maintain uniformity among the large number of models contained within Web-ICE, the "N-fold" difference among each estimated and actual value was calculated and used to determine the fitness of the estimated toxicity value. For aquatic species, inter-laboratory variation of acute toxicity test data for a given species and chemical can be as great as a 5-fold difference (Fairbrother 2008). For wildlife species, the average range of multiple toxicity measurements for a specific chemical and species was determined to be between 4.0 and 6.4 (Raimondo et al. 2007). Thus, a 5-fold difference was deemed a good fit in the validation analysis of both aquatic and wildlife models.

The cross-validation success rate was calculated for each model as the proportion of removed data points that were predicted within 5-fold of the actual value from models that were statistically significant. In cases where the removal of a xy data pair resulted in the development of a model that was not significant at the $p < 0.05$ level, these replicates were not included in the cross-validation success rate. This is because models that are not significant at the $p<0.05$ level have a greater risk of Type I error. This was only the case for models with low degrees of freedom (<8) and a p-value between 0.01 and 0.05 in the original model.

There is a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty increasing with larger taxonomic distance (Raimondo et al., 2007). In aquatic species, models predict within 5-fold and 10-fold of the actual value with 91 and 96% certainty for surrogate and predicted taxa within the same family, and for 86 and 96% within the same order. In wildlife species, models predict within 5-fold and 10-fold of the actual value with 90 and 97% certainty for surrogate and predicted taxa within the same order. Model certainty decreases with increasing taxonomic distance. A more detailed account of model uncertainty as it relates chemical mode of action/class is discussed in Raimondo et al. (2007).

Using the Web-ICE Program

The Web-ICE platform contains separate modules that predict acute toxicity to aquatic (vertebrates and invertebrates) species, genera, or families (**ICE Aquatic**) and wildlife (terrestrial birds and mammals) species or families (**ICE Wildlife**) (Figure 1). The **Species Sensitivity Distribution Module** is available for aquatic and wildlife species and batch processes species level toxicity from all entered surrogates. The **Endangered Species Module**, also available for aquatic and wildlife taxa, predicts toxicity to listed species from all available species, genus, or family level models for the entered surrogates. Each module is accessible from either the home page or from the blue navigation bar along the left side of the page. Before working with a Web-ICE module, you must first decide if you are going to work with aquatic or wildlife taxa, the program does not contain models that estimate wildlife toxicity from an aquatic surrogate, or vice versa.

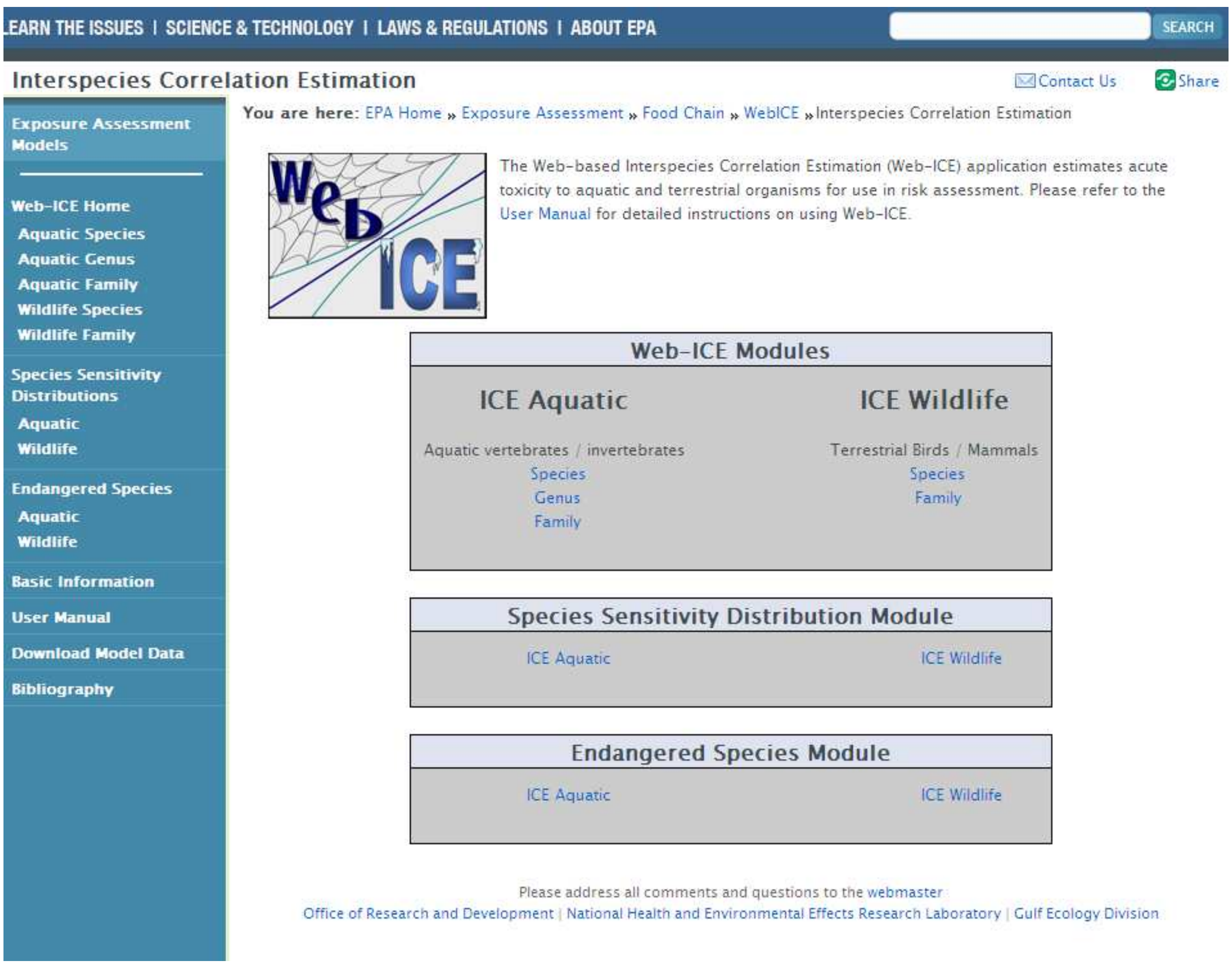

Figure 1. Home page of Web-ICE program

I. Working with Web-ICE Aquatic or Web-ICE Wildlife Modules

Selecting Model Taxa

1. From either the home page or the blue navigation bar, click the link for the module with which you will be working (Aquatic species, genus, or family; Wildlife species or family).
2. You will then be directed to a **Taxa Selection Page** (Figure 2) which will allow you to select your surrogate and predicted taxa for the model.
3. You may search for your surrogate and predicted taxa by either common name or scientific name by selecting the appropriate option in the **Sort by:** drop down menu. The default is set to common name.
4. From the drop down menus, select the surrogate species and predicted taxon. It does not matter which you select first; however, the second choice is limited to the models available for the taxon chosen first.
5. To change any of your selections, press **Reset** and start again.
6. Click **Continue** to be directed to the calculator page for toxicity estimation.

If there is not a model for your predicted species of interest, you will need to use a genus or family-level model to predict toxicity. The available models may be determined by browsing through the genus (aquatics only) and family level modules, or by searching through the spreadsheets of model information available through the **Download Model Data** option on the blue navigation bar. The downloadable Microsoft Excel® spreadsheets provided for each Web-ICE module may be sorted by surrogate species or predicted taxa to identify available models.

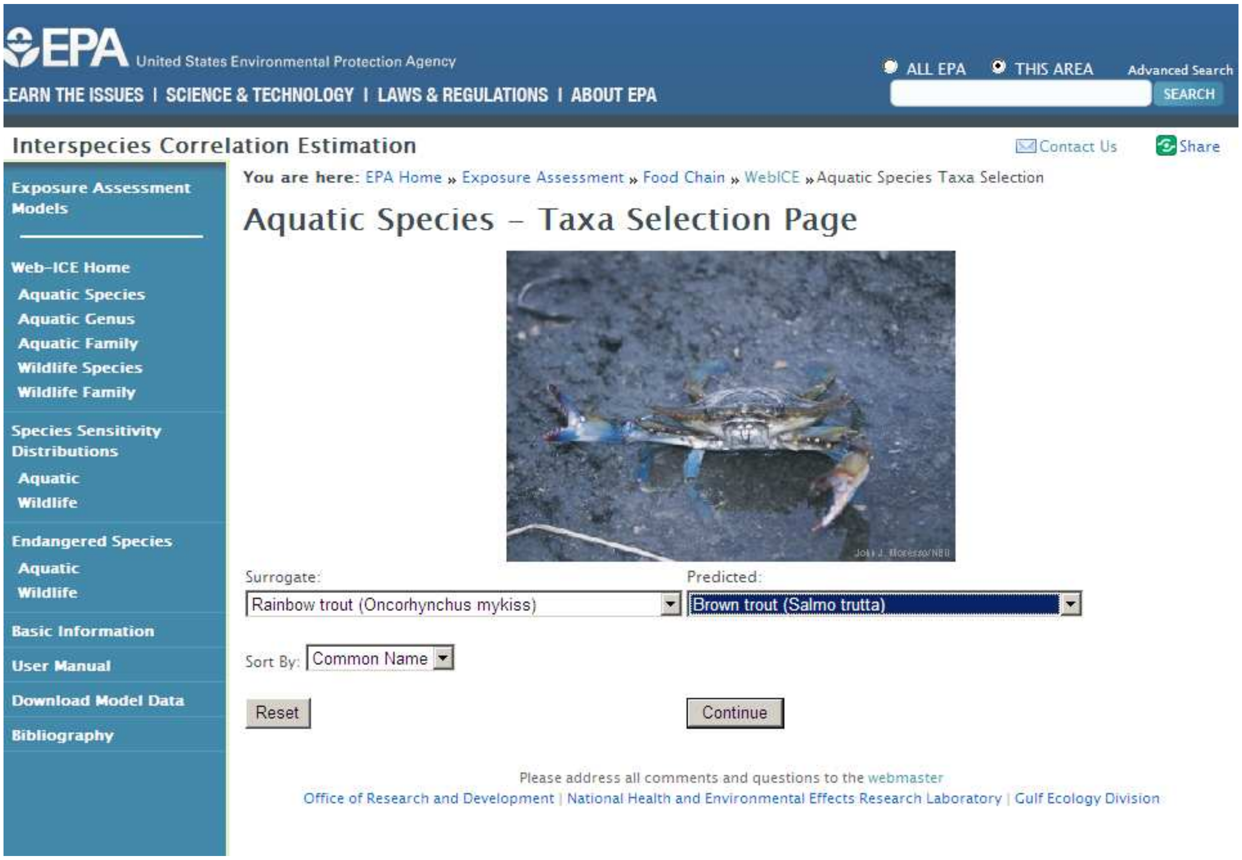

Figure 2. Taxa selection page

Estimating Toxicity

The surrogate and predicted species selected from the previous page are listed at the top of a calculator page (Figure 3). This page is divided into four parts: input, calculated results, model statistics, and model graphic. The known toxicity for the surrogate species is entered under **Surrogate Acute Toxicity**, below which the desired confidence limits can be selected (Figure 3A). Predicted toxicity estimates and confidence intervals are displayed under **Predicted Acute Toxicity** (Figure 3B). The bottom left side of the page contains the model statistics (Figure 3C). Please refer to the *Statistical Definitions* section of this manual for more specific information. The graph shows the data (LC50/LD50 values) used to develop the model, the regression line (straight inner line), and 95% confidence intervals (curved outer lines) (Figure 3D). The surrogate and predicted taxa are labeled on the X and Y axes, respectively. Both the model statistics and the graph are unique for each model and will change for each surrogate species and predicted taxon.

1. Enter the acute toxicity value in the box located under **Surrogate Acute Toxicity** (Figure 3A).
2. Select your desired confidence interval (90, 95, or 99%) from the drop down menu located under **Select Confidence Interval** (Figure 3A). The default for the confidence intervals is 95%.

3. Press **Calculate**
4. The calculated values will appear in the three boxes labeled **Predicted Acute Toxicity**, **Lower Limit** and **Upper limit** (Figure 3B).
5. Log-transformed values of the surrogate and predicted toxicity values appear in parentheses next to the values.
6. If the entered surrogate toxicity value is outside the range of toxicity values used to develop the model, a pop-up with the warning "**This value is outside the x-axis range for this model. Continue?**" will appear. The user may select "OK" to proceed to calculate the toxicity value or hit cancel to enter another value.
7. To select a different model, select the link to the desired module in the blue navigation bar on left side of the page.

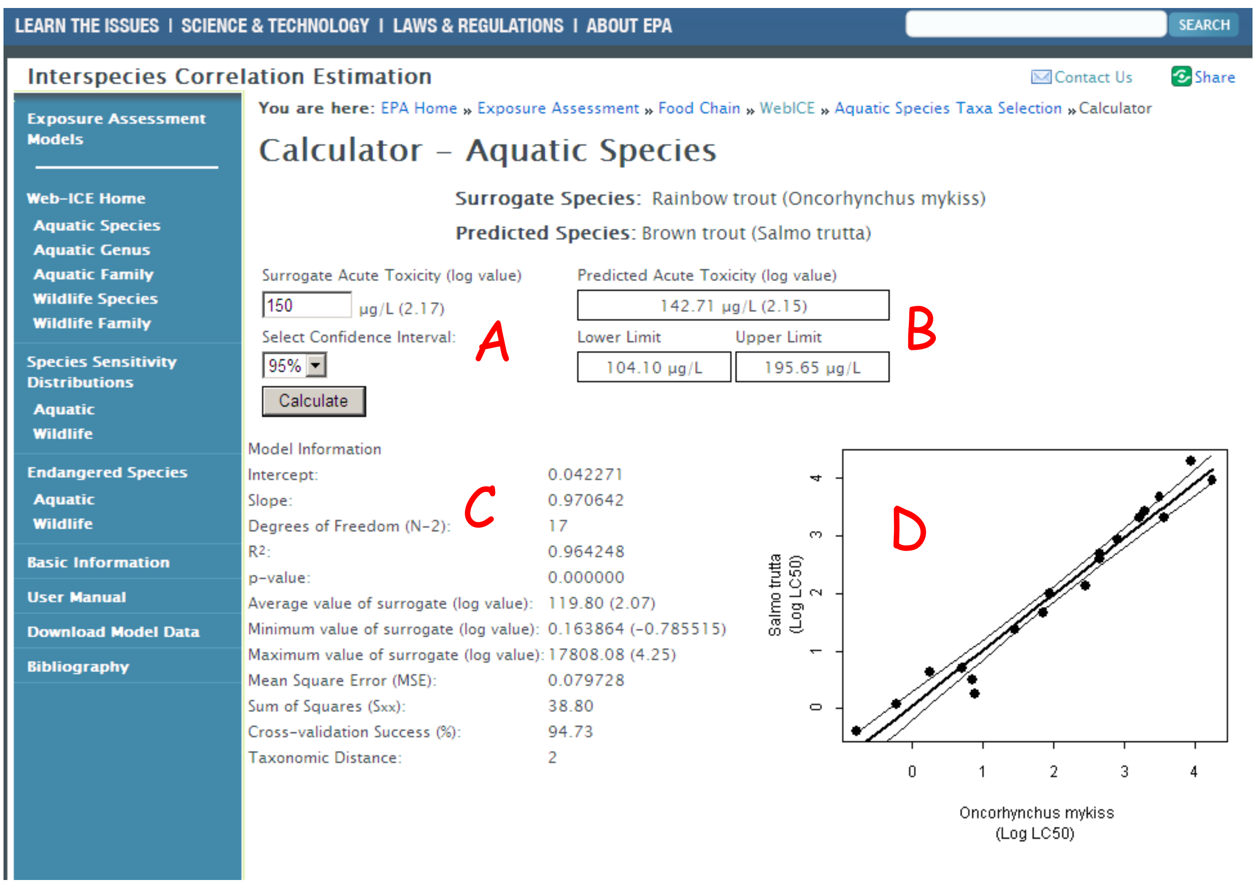

Figure 3. Calculator Page

II. The Species Sensitivity Distribution (SSD) Module

Species Sensitivity Distributions (SSDs) are probabilistic models that describe the sensitivity of biological species to a chemical. SSDs generated in Web-ICE are log-logistic cumulative distribution functions of toxicity values for multiple species (de Zwart 2002) and are used to estimate a hazard level (hazardous concentration (HC) or hazardous dose (HD)) that is protective of most test species (e.g., 95%) by estimating the concentration or dose at a corresponding percentile (e.g., 5^{th}) of the distribution (Dyer et al. 2006).

The SSD modules for aquatic and wildlife species generate SSDs from Web-ICE toxicity values estimated from one or more surrogate species. Toxicity values for one or more surrogate species are used to simultaneously estimate toxicity to all possible predicted species with existing Web-ICE models. The SSD is then generated using all estimated toxicity values and the entered toxicity of the surrogate species. Toxicity values for up to 25 surrogate species may be entered (Figure 4). If more than one surrogate species estimates toxicity to the same predicted species, Web-ICE selects the toxicity value with the smallest confidence intervals. If multiple surrogates are used and a predicted value is estimated for one of the surrogate species, Web-ICE uses the entered value for that species and excludes the predicted value(s) from the SSD.

An HC/HD level is automatically calculated from the distribution. The user can deselect toxicity values for predicted species that they wish to exclude from the SSD by clicking on the box to the left of the predicted species (Figure 5), and the associated HC/HD value is automatically recalculated. An HC/HD drop down menu on the output page allows the user to specify the hazard level to calculate. **HC1/HD1** corresponds to the 1^{st} percentile, **HC5/HD5** corresponds to the 5^{th} percentile, and **HC10/HD10** corresponds to the 10^{th} percentile. The default is set to HC5 for aquatic species and HD5 for wildlife species.

Web-ICE uses the SSD described by the logistic distribution function of de Zwart (2002):

$$F(C) = 1/(1 + \exp((\alpha - C) / \beta))$$

The $\log_{10}$-transformed environmental concentration (or dose) of the evaluated chemical is represented by C, the parameter, α, is the sample mean of the $\log_{10}$ -transformed toxicity values and β is defined as $\sqrt{3}/\pi * \sigma$, where σ is the standard deviation of the $\log_{10}$ -transformed toxicity values (de Zwart 2002). The HC/HD level is determined as the percentile of interest (e.g., 5^{th}) of the described distribution. Corresponding SSDs are also developed from the upper and lower confidence limits of the predicted toxicity values and are used to calculate the upper and lower bounds of the HC/HD value at a given percentile. For example, the lower bound of the HC5 is calculated as the 5^{th} percentile of the SSD developed from the estimated lower confidence limit of each predicted toxicity value. Similarly, the upper bound of an HC5 is calculated as the 5^{th} percentile of the SSD developed from the estimated upper limit of each predicted toxicity value.

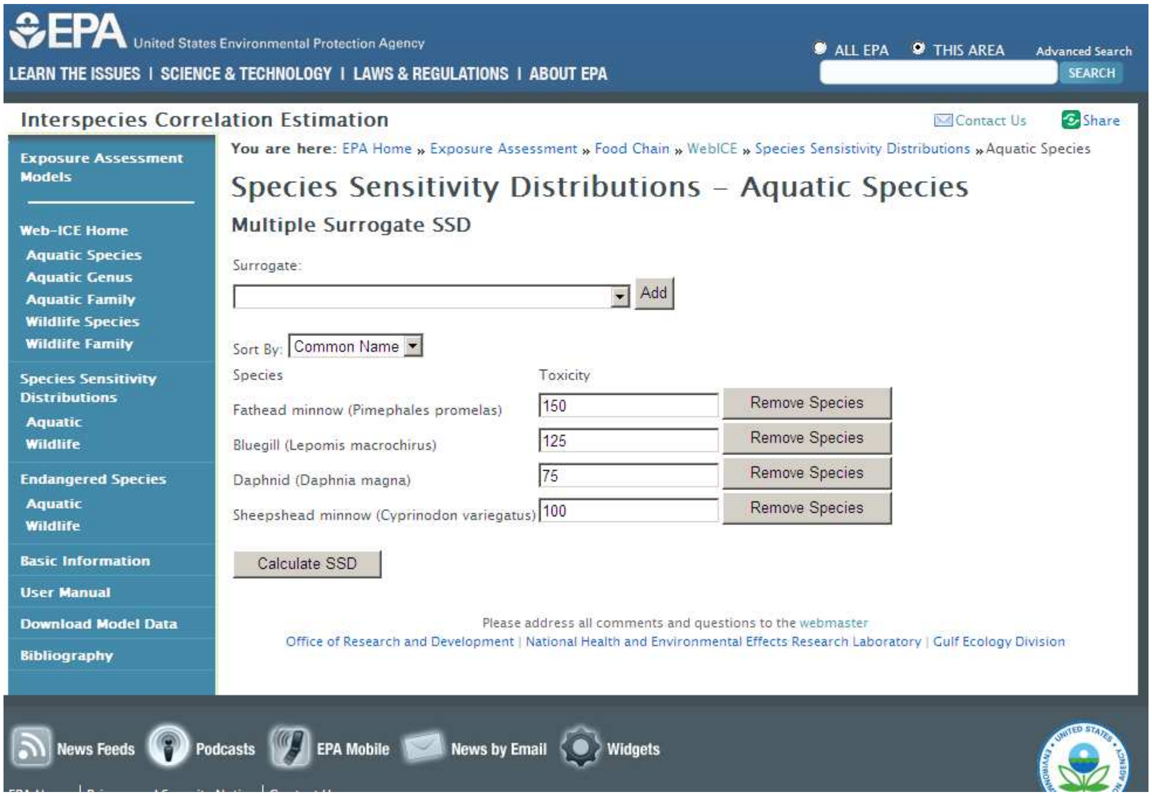

Figure 4. SSD taxa selection page.

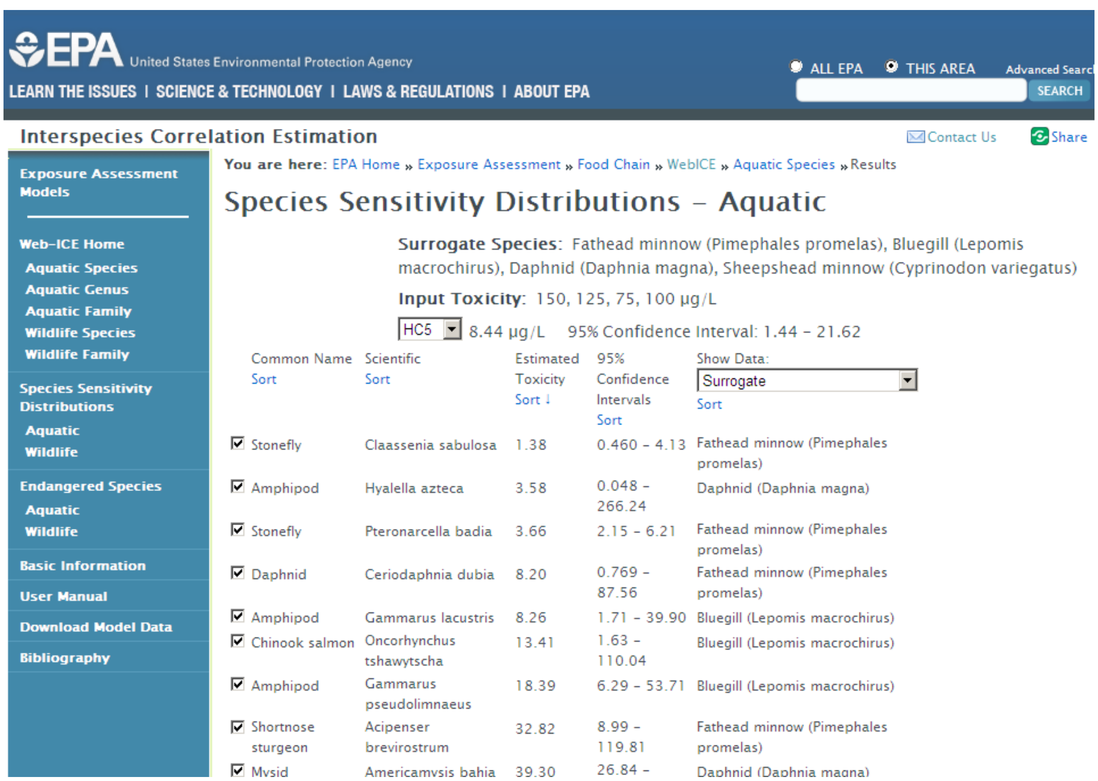

Figure 5. SSD output page.

Generating an SSD:

1. Under the SSD module, select either Aquatic or Wildlife.
2. On the SSD taxa selection page, select your surrogate species from the drop down menu and click **Add** to add the species as a surrogate.
3. If desired, select additional surrogate species from the drop down menu and click **Add**. A maximum of 25 species can be selected.
4. To remove a surrogate species from the list after it is added, click **Remove** next to the species name.
5. Enter the known toxicity for the surrogate species, click **Calculate SSD**.
6. On the SSD output page, the HC/HD level may be changed from the drop down box. The hazard level is automatically recalculated if the level is changed. The default is the HC/HD5.
7. The warning "**Input toxicity is greater (less) than model maximum (minimum)"** indicates if a predicted value was generated from a surrogate species toxicity value that was outside the range of toxicity values used to generate that model.
8. The user can unmark the box to the left of a predicted species to exclude it from the SSD, which is automatically recalculated. (NOTE: See *Selecting Predicted Toxicity Values for SSDs* in the *Guidance for Model Selection and Use* section below for guidance on removing estimated toxicity values).
9. The drop down menu in the **Show Data** column provides additional model information (surrogate, taxonomic distance, cross-validation success rate, degrees of freedom, R^2, p-value, or mean square error) for the user to view.
10. The user may sort the ICE-estimated toxicity values by each column by selecting the **sort** tab below the column heading.

III. The Endangered Species Module

The Endangered Species Module batch processes toxicity values for endangered species from all species, genus, and family level models available for the entered surrogates. The list of threatened and endangered species was obtained from the US Fish and Wildlife Service Threatened and Endangered Species module of Environmental Conservation Online System (http://ecos.fws.gov/tess_public; Accessed August 2007), which was linked to Web-ICE species, genus, and family model databases for aquatic organisms and wildlife. Users may predict to all available endangered species within a broad taxonomic groups (e.g., Fishes) or a particular species (e.g., Atlantic Salmon, *Salmo salar*) using up to 25 surrogates.

Producing an Endangered Species Toxicity Report

1. Under the Endangered Species module, select either Aquatic or Wildlife.

2. On the Endangered Species taxa selection page, select either the broad taxa of interest (e.g., Fishes) or a particular species of interest from the drop down menu (Figure 6).
3. Select your surrogate species from the drop down menu and click **Add** to add the species as a surrogate. A maximum of 25 species can be selected.
4. To remove a surrogate species from the list after it is added, click **Remove** next to the species name.
5. Enter the known toxicity for the surrogate species, click **Calculate**.
6. The Endangered species output page provides the estimated toxicity for each predicted taxa, the model level (e.g., species), surrogate, and model information (Figure 7).
7. The user may sort the ICE-estimated toxicity values by each column by selecting the **sort** tab below the column heading.

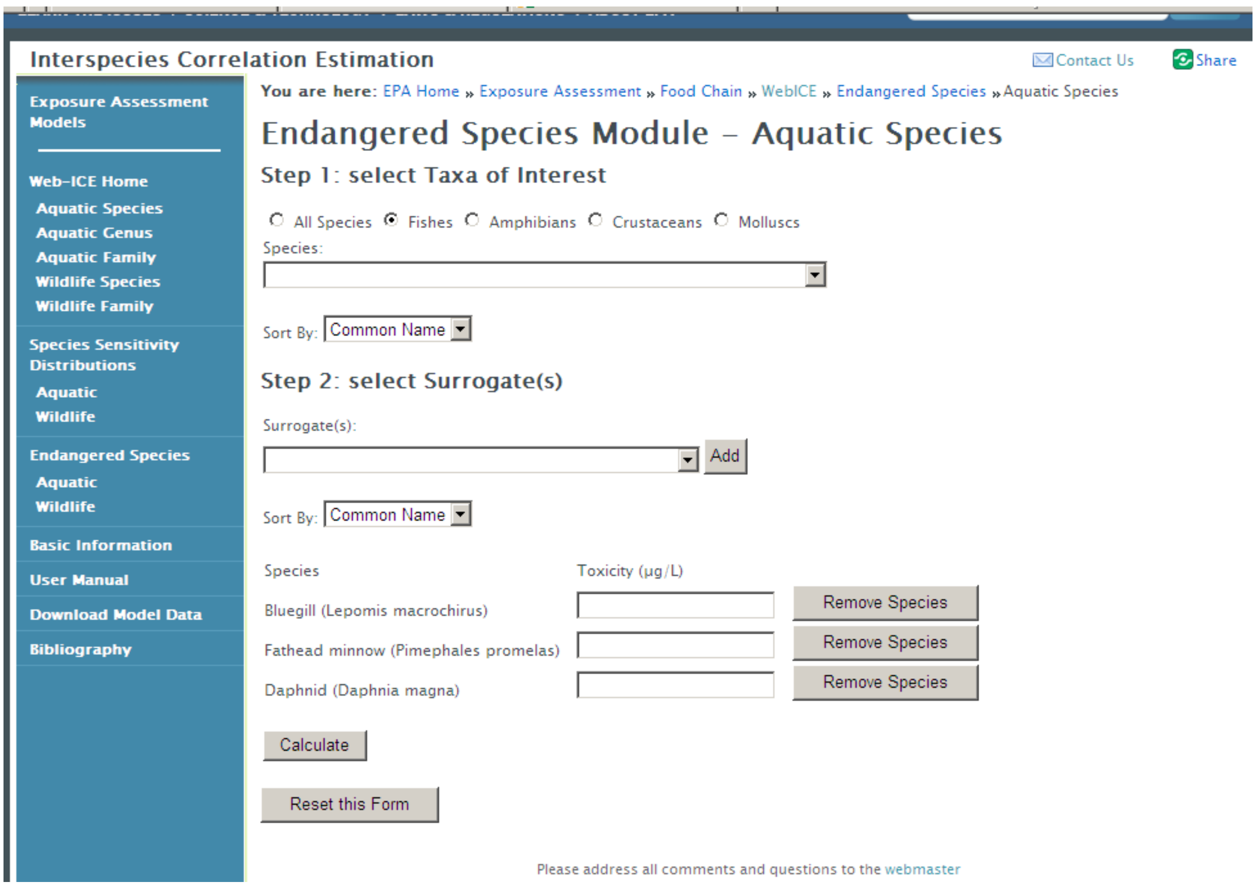

Figure 6. Taxa selection page of Endangered Species module.

You are here: EPA Home » Exposure Assessment » Food Chain » WebICE » Aquatic Species » Results

Endangered Species – Aquatic

Surrogate Species: Fathead minnow (Pimephales promelas), Bluegill (Lepomis macrochirus), Rainbow trout (Oncorhynchus mykiss), Sheepshead minnow (Cyprinodon variegatus)

Input Toxicity: 500, 400, 200, 300 µg/L

Predicted Taxa Sort ↓	Model Level Sort	Surrogate Sort	Estimated Toxicity Sort	95% Confidence Intervals Sort	Degrees of Freedom (N-2) Sort	R2 Sort	p-value Sort	Mean Square Error (MSE) Sort	Cross-validation Success (%) Sort	Taxonomic Distance Sort
Chinook salmon (Oncorhynchus tshawytscha)	species	Bluegill (Lepomis macrochirus)	64.03	13.27 - 308.87	4	0.9039	0.0035	0.2084	83.33	4
Chinook salmon (Oncorhynchus tshawytscha)	species	Rainbow trout (Oncorhynchus mykiss)	286.82	171.75 - 478.99	6	0.9793	0.0000	0.0644	100.00	1
Oncorhynchus	genus	Fathead minnow (Pimephales promelas)	251.35	182.06 - 347.01	84	0.8328	0.0000	0.3636	84.88	4
Oncorhynchus	genus	Bluegill (Lepomis macrochirus)	336.31	296.33 - 381.69	310	0.8840	0.0000	0.2215	90.38	4
Oncorhynchus	genus	Rainbow trout (Oncorhynchus mykiss)	235.62	196.89 - 281.98	45	0.9572	0.0000	0.0721	97.87	1
Oncorhynchus	genus	Sheepshead minnow (Cyprinodon variegatus)	243.06	157.32 - 375.54	73	0.6632	0.0000	0.5161	78.66	4
Salmonidae	family	Fathead minnow (Pimephales promelas)	241.68	180.64 - 323.35	86	0.8565	0.0000	0.3022	87.5	4
Salmonidae	family	Bluegill (Lepomis macrochirus)	333.60	294.35 - 378.08	312	0.8852	0.0000	0.2177	91.08	4
Salmonidae	family	Rainbow trout	230.15	196.48 -	55	0.9555	0.0000	0.0683	98.24	2

Figure 7. Endangered species predicted toxicity report

IV. Accessing Model Data

A list of chemicals in the aquatic and wildlife databases is available for download using the **Chemicals in Aquatic** and **Chemicals in Wildlife** links. In the **Chemicals in Aquatic** file the chemical CAS number and associated toxicity values used in each model are provided. The **Chemicals in Wildlife** file contains the number of species present for each chemical. The acute data used to develop the ICE models for wildlife are not available due to proprietary rights of some information.

Models for all Web-ICE aquatic and wildlife modules are available as a downloadable Microsoft Excel® spreadsheet under the **Download Model Data** option on the blue navigation bar. The data spreadsheets include model parameters (R^2, p-value, df, intercept, slope, standard error of the slope, Sxx, and MSE), general model information (taxonomic distance, cross-validation success rate), descriptive statistics (average, minimum, and maximum values of the surrogate species), and critical t-values used to calculate 90, 95, and 99% confidence intervals (t90, t95, t99). These spreadsheets provide all of the information that is needed to generate Web-ICE toxicity estimates and confidence intervals, as well as facilitate the selection of the most robust models.

Using model data provided, users may calculate toxicity as:

$$\text{Predicted toxicity} = 10\text{\^{}}(\text{intercept} + \text{slope} * \text{Log}_{10}(\text{surrogate toxicity}))$$

And confidence intervals as:

$$\text{Lower bound} = 10^\wedge(\log(\text{predicted}) - t_{1-\alpha} * \sqrt{[\text{MSE}*(1/n + (\log(x) - x.\text{ave})^\wedge 2/\text{Sxx})\ ]})$$
$$\text{Upper bound} = 10^\wedge(\log(\text{predicted}) + t_{1-\alpha} * \sqrt{[\text{MSE}*(1/n + (\log(x) - x.\text{ave})^\wedge 2/\text{Sxx})\ ]})$$

Where x is the untransformed value of surrogate toxicity, x.ave is the average value of log-transformed surrogate toxicity values, Sxx is the sum of squared deviations of the surrogate, MSE is the mean square error, and $t_{1-\alpha}$ is the value of the t distribution corresponding to the desired level of confidence (ie. 90, 95, 99%).

Guidance for Model Selection and Use

I. Statistical Definitions

Several statistics are provided with each model and may be used to evaluate the accuracy and precision of the estimated value. These statistics are shown to the left of the graph on the calculator page (Figure 3C) and are provided in the spreadsheet of model information available in the **Download Model Data** option. The following provides a basic interpretation of model statistics to help guide users in model selection:

Intercept – The $\log_{10}$ value of the predicted taxon toxicity when the $\log_{10}$ of the surrogate species toxicity is 0.

Slope – The regression coefficient represents the change in $\log_{10}$ value of the predicted taxon toxicity for every change in $\log_{10}$ value of the surrogate species toxicity.

Degrees of Freedom (df, N - 2) – The number of data points used to build the model minus two. Degrees of freedom are related to statistical power; in general, the higher the degrees of freedom, the more robust the model.

R^2 – The proportion of the data variability that is explained by the model. The greater the R^2 value and the closer it is to one, the more robust the model is in describing the relationship between the predicted and surrogate taxa.

p-value – The significance level of the linear association and the probability that the linear association was a result of random data. Models with lower p-values are more robust. Model p-values of < 0.00001 are reported as 0.00000.

Average value of the surrogate – The average of toxicity values for the surrogate species used in the model. The first number is the actual value and the number in parentheses is the log-transformed value.

Minimum value of the surrogate – The lowest toxicity value for the surrogate species used in the model. The first number is the actual value and the number in parentheses is the log-transformed value.

Maximum value of the surrogate – The largest toxicity value for the surrogate species used in the model. The first number is the actual value and the number in parentheses is the log-transformed value.

Mean Square Error (MSE) – An unbiased estimator of the variance of the regression line.

Sum of Squares (Sxx) – Sum of squared deviations of the surrogate.

Cross-validation Success – The percentage of removed data points that were predicted within 5-fold of the actual value. Models with a Cross-validation Success of "na" are those that either had df = 1 or where no significant models were developed when data points were removed.

Taxonomic Distance – The taxonomic relationship between the surrogate and predicted taxa. Two taxa within the same genus have taxonomic distance of 1; within the same family = 2; within the same order = 3; within the same class = 4; within the same phylum = 5; within the same kingdom = 6.

II. Selecting a Model with Low Uncertainty

Rules of Thumb

Model attributes, such as taxonomic distance of the predicted and surrogate species, model parameters (listed below) and cross-validation success rate, should be used to select models with low uncertainty. For best estimates, models should be selected that possess the following:

1. Relatively low mean square error (MSE) (<0.22)
2. Close taxonomic distance ($\leq$ 3)
3. High cross-validation success rate (> 85%)
4. High degrees of freedom (df > 8, N > 10)
5. High R^2 value (> 0.6)
6. Low p-values (< 0.01)
7. Narrow confidence bands on the graph

The best estimations generally occur for surrogate and predicted taxa that are within the same genus, family, or order and for models with R^2 > 0.6 (Raimondo et al. 2007). In general, models with more degrees of freedom (df) have greater statistical power and choosing a model with df greater than 8 is recommended to reduce model uncertainty. A priori power analysis determined that linear models with df > 8 have enough statistical power (1-ß > 0.8) to sufficiently increase the chance of finding a significant relationship within the data. It is also recommended to choose models with p-values < 0.01 to further reduce the chance of Type I errors in the toxicity estimations.

Cross-validation success rate is a conservative estimate of model uncertainty and should not be interpreted as an exact estimate of model error. Cross-validation removes data from the original model, potentially causing a large change in the model for small datasets. Due to changes in a model (i.e. reduced df, altered slope/intercept) during this validation process, cross-validation success rate should be considered only an estimate of generalization error. Particularly for models built from small datasets, actual error can be expected to be lower than cross-validation error.

Surrogate Species Selection: An Example

In an example of how to select a suitable model, Raimondo et al. (2007) outlined a selection procedure to find an appropriate surrogate species to estimate the toxicity of a chemical to red-winged blackbird. In the example, toxicity data for the chemical of interest was available for northern bobwhite, mallard, Japanese quail, fulvous whistling duck, common grackle, and house sparrow, making them all potential surrogates. The common grackle and house sparrow have the closest taxonomic distance (2, same family; 3, same order); the other potential surrogates in this example have a taxonomic distance of 4 (same class). Of the grackle and house sparrow, both have similar MSE (~0.13), however house sparrow has a higher model R^2 (0.84), higher cross-validation success rate (95), and greater degrees of freedom (107), and is the best surrogate for red-winged blackbird in this example. The grackle would also provide good surrogacy, with high R^2 (0.65), high cross-validation success rate (93), and good degrees of freedom (54). If neither of these species were available surrogates, Japanese quail (R^2 = 0.79, MSE = 0.15, df = 135, cross-validation success rate = 91) would be the next best surrogate, followed by northern bobwhite (R^2 = 0.63, MSE = 0.23, df = 45, cross-validation success rate = 85) and mallard (R^2 = 0.48, MSE = 0.34, df = 80, cross-validation success rate = 79). Although fulvous whistling duck has the highest model R^2, low degrees of freedom (df = 2) and comparatively higher MSE (0.30) do not make it as suitable of a surrogate as the other species.

III. Evaluating Model Predictions

Uncertainty of model predictions may be evaluated by assessing (1) the characteristics of the model used in the predictions, and (2) the value of the input data relative to the data used to generate the model. The former was discussed in the previous section and the *Rules of Thumb* should be followed to ensure high confidence in model selection. Even for robust models, however, model uncertainty increases outside the range of surrogate species toxicity values that were used to develop the model.

Uncertainty may be evaluated by reviewing the confidence intervals calculated with the predicted value. Narrow confidence intervals represent higher confidence that the model fits through the range of datapoints for the entered surrogate species toxicity. If the surrogate toxicity value entered into an ICE model is outside the range of surrogate toxicity data used to generate the model, the warning "**This value is outside the x-axis range for this model. Continue?"** will appear to alert the user. This warning alone does not indicate low confidence in the model estimate, but should be used in conjunction with the calculated confidence intervals to evaluate the model prediction. For example, if the upper and lower bounds of the confidence interval are several orders of magnitude from the predicted value, caution should be used in applying the ICE estimate in risk assessment.

IV. Selecting Predicted Toxicity Values for SSDs

The SSD modules of Web-ICE automatically predict toxicity values from all available models for the selected surrogate species simultaneously. The user has the discretion to remove predicted toxicity values from the SSD to either customize the SSD for a particular taxa (e.g., birds only, fish only), or to remove predicted toxicity values with large confidence intervals. If an estimated toxicity value was derived from an input value that was outside of the range of surrogate species data used to generate the model from which it was predicted, a warning appears next to the value indicating the maximum or minimum value of the model. This warning alone does not indicate low confidence in the model estimate, but should be used in conjunction with the calculated confidence intervals to evaluate the model prediction.

Users should also use the confidence intervals around the HC/HD level to guide the selection of toxicity values to exclude from the SSD. Cases in which the upper bound of the SSD is less than the HC/HD level occur when predicted toxicity values with extremely large confidence intervals are included in the SSD; removal of predicted toxicity with such confidence intervals results in HC/HD values with adequate confidence. Users may also refer to the model information provided by the **Show Data** drop down menu when selecting data to include in SSDs.

V. Applying Web-ICE in Ecological Risk Assessment (ERA)

Web-ICE was developed to support both chemical hazard assessment and ecological risk assessment (ERA) by providing a method to estimate acute toxicity to specific taxa, such as endangered species, or to a larger number of taxa (species, genera, families) with known uncertainty. Potential applications of acute toxicity values generated by Web-ICE include the problem formulation phase of an ERA to screen for contaminants of potential concern and in the analysis phase to characterize effects to a larger number of species. The estimation of species-specific toxicity values using Web-ICE is recommended as an alternative to safety factors typically applied when extrapolating toxicity or risks to taxa without chemical and species-specific toxicity data. Another potential application of the chemical and taxon-specific acute toxicity estimates generated from ICE models include input into existing exposure and risk models (e.g., TREX; EPA 2005). Web-ICE generated toxicity values may also be used in the analysis of uncertainty and variability in toxicity to ecological receptors in both screening level and baseline or Tier II ERAs.

In the absence of taxa-specific ICE models, Web-ICE can be used to generate SSDs and estimated 1st, 5th or 10th percentile values of the cumulative distribution of species-specific toxicity values. These percentile values, expressed as the hazard concentration (e.g., HC5) or hazardous dose (e.g., HD5), provide an estimate of toxicity at a prescribed level of species protection with known uncertainty. Hazard concentrations could be used in ERA in place of species-specific toxicity values or as a component of the uncertainty analysis.

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References

American Society for Testing and Materials (ASTM). 2007. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E 729-96(2007). Philadelphia PA.

Asfaw, A., M. R. Ellersieck, and F. L. Mayer. 2003. Interspecies Correlation Estimations (ICE) for acute toxicity to aquatic organisms and wildlife. II. User Manual and Software. EPA/600/R-03/106. U.S. Environmental Protection Agency, National health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. 14 p.

Awkerman, J., S. Raimondo, and M.G. Barron.2008. Development of Species Sensitivity Distributions for wildlife using interspecies toxicity correlation models. Environ. Sci. Technol. 42 (9): 3447-3452.

Baril, A., B. Jobin, P. Mineau, and B. T. Collins. 1994. A consideration of inter-species variability in the use of the median lethal dose (LD_{50}) in avian risk assessment. Technical Report No. 216. Canada Wildlife Service, Headquarters.

De Zwart, D. 2002. Observed regularities in species sensitivity distributions for aquatic species. In Species Sensitivity Distributions in Ecotoxicology, L. Posthuma, G.W. Suter, T.P.Traas, Eds. Lewis Publishers, Boca Raton, FL. pp133-154.

Dyer, S. D., D. J. Versteeg, S. E. Belanger, J. G. Chaney, and F. L. Mayer. 2006. Interspecies correlation estimates predict protective environmental concentrations. Environ. Sci. Technol. 40: 3102-3111.

Dyer, S. D., D. J. Versteeg, S. E. Belanger, J. G. Chaney, S. Raimondo and M. G. Barron. 2008. Comparison of Species Sensitivity Distributions Derived from Interspecies Correlation Models to Distributions used to Derive Water Quality Criteria. Environ. Sci. Technol. 42: 3076-3083.

Fairbrother, A. 2008. Risk Management Safety Factor. In. Encyclopedia of Ecology, vol. 4. S. E. Jørgensen and B. D. Fath (eds.). Elsevier publishing. pp. 3062-3068.

Hudson, R. H., R. K. Tucker, and M. A. Haegele. 1984. Handbook of toxicity of pesticides to wildlife. U.S. Fish and Wildlife Service, Resource Publ. 153, Washington D.C. 90 p.

Insightful. 2001. S-plus 6 Guide to Statistics. Volume 1. Insightful Corporation, Seattle, WA.

Mayer, F. L. and M. R. Ellersieck. 1986. Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals. US Fish and Wildlife Service Resource Publication 160. Washington DC. 579 p.

Mineau, P., A. Baril, B. T. Collins, J. Duffe, G. Joerman, and R. Luttik. 2001. Pesticide acute toxicity reference values for birds. Rev. Environ. Contam. Toxicol. 170: 13-74.

Raimondo, S., P. Mineau, and M. G.Barron. 2007. Estimation of chemical toxicity in wildlife species using interspecies correlation models. Environ. Sci. Technol. 41: 5888-5894.

Raimondo, S., D.N. Vivian, C. Delos, M.G. Barron. 2008. Protectiveness of Species Sensitivity Distribution Hazard Concentrations for Acute Toxicity Used in Endangered Species Risk Assessment. Environ. Toxicol. Chem. 27 (12): 2599-2607.

Raimondo, S., D.N. Vivian, and M.G. Barron. 2009. Standardizing acute toxicity data for use in ecotoxicological models: influence of test type, life stage, and concentration reporting. Ecotoxicology. 18: 918-928.

Shafer, E. W. Jr. and W. A. Bowles Jr. 1985. Acute oral toxicity and repellency of 933 chemicals to house and deer mice. Arch. Environ. Contam. Toxicol.14: 111-129.

Shafer, E. W. Jr. and W. A. Bowles Jr. 2004. Toxicity, repellency or phototoxicity of 979 chemicals to birds, mammals and plants. Research Report No. 04-01. United States Department of Agriculture, Fort Collins, CO. 118 p.

Shafer, E. W. Jr., W. A. Bowles Jr. and J. Hurlbut,. 1983. The acute oral toxicity, repellency and hazard potential of 998 chemicals to one or more species of wild and domestic birds. Arch. Environ. Contam. Toxicol. 12: 355-382.

Smith, G. J. 1987. Pesticide use and toxicology in relation to wildlife: organophosphorus and carbamate compounds. Resource Publication 170. United States Department of the Interior, Washington, DC. 171 p.

US Environmental Protection Agency (EPA). 1986. Quality criteria for water. EPA 440/5-86-001. Washington, DC.

US Environmental Protection Agency (EPA). 1996. Ecological Effects Test Guidelines. OPPTS 850.1075 Fish Acute Toxicity Test, Freshwater and Marine. EPA 712-C-96-118. Washington DC.

US Environmental Protection Agency (EPA). 2005. TREX: Terrestrial Residue EXposure model. Office of Pesticide Programs. U.S. Environmental Protection Agency.
http://www.epa.gov/oppefed1/models/terrestrial/trex_usersguide.htm#content4

US Environmental Protection Agency (EPA). 2006. ECOTOX Ecotoxicology Database. http://cfpub.epa.gov/ecotox. Duluth MN.

Appendices

Appendix I. Summary of acceptance requirements for data included in ICE models

Component	Information required	Acceptance requirements
Test organism	Aquatic taxa tested	fish, aquatic invertebrates, amphibians
		species level model: identifiable to genus and species
		genus or family level model: identifiable to genus or family
	Life stage[1]	juvenile only: fish, amphibians, insects, mollusks, decapods
		all life stages: all other species
	Salinity requirements	identifiable as freshwater (FW) or saltwater (SW; estuarine or marine) organism
Test chemical	Test chemical identity	reported CAS, chemical name or structure
		confirmed name and CAS
	Test chemical purity	≥90% or analytical/reagent grade or equivalent
	Single compound tested[2]	CAS corresponds to single compound or element
		mixtures excluded except for chemical salts and specific congener mixtures
Test conditions	Aqueous exposure	no sediment, dietary or mixed dose exposures
		no phototoxicity results
	Test duration	48 hr: daphnids, midges, mosquitoes
		96 hr: all other species
	Test type	static, flow-through or static renewal
	Temperature[3]	species specific (± 3C)
	Dissolved oxygen[3]	Test type specific[4]
	Salinity[3]	<1 ppt: FW species[5]
		1-5ppt: *Cyprinodon bovinus*
		≥15 ppt: SW species[6]

Component	Information required	Acceptance requirements
	pH or hardness (FW only: required for specific chemical normalizations)	pH: ammonia, pentachlorophenol (PCP)
		Hardness: Ag, Cu, Cd, Cr(III), Pb, Ni, Zn
Reported toxicity value	Acute toxicity endpoint: death (LC50) or immobilization (EC50)	48 hr EC50/LC50: daphnids, midges, mosquitoes
		96 hr EC50/LC50: all other invertebrates
		96 hr LC50: fish, amphibians
	Concentration units	mass/volume or molar units
Toxicity value standardization	Concentration units	conversion to ug/L
	Chemical specific normalizations[7]	PCP: pH 6.5
		ammonia: pH 8; temperature dependent
		Ag, Cu, Cd, Cr(III), Pb, Ni, Zn: hardness 50 mg/L
	Element specific normalization[7]	Ag, Al, Cu, Cd, Co, Cr(III), Cr(VI), Hg, NH4, Ni, Pb, Zn

1. If life stage not reported, must be determined through reported age/size.
2. Only tests of single compounds; included metal and other chemical salts, and specific congener mixtures (e.g., standard Aroclors, toxaphene).
3. Meets ASTM or equivalent test guidelines for test species.
4. Test type specific dissolved oxygen saturation. Static: ≤48 hr 60-100%; >48 hr 40-100%. Static renewal or flow-through: 60-100%.
5. FW: test water source identifiable as freshwater, reported salinity <1 ppt, or test species is a stenohaline freshwater species; only FW salmonid tests.
6. SW: test water identifiable as saltwater, salinity reported to be ≥ 15 ppt, or test species is a stenohaline saltwater species; only SW striped bass tests were included.
7. Normalized according to AWQC.

Appendix II. List of Species in Aquatic Database

The following species were used to develop Web-ICE aquatic species, genus, or family-level models.

Invertebrates

		Platyhelminthes	
Tricladida			
	Planariidae	*Dugesia tigrina*	Flatworm
		Annelida	
Aciculata			
	Nereididae	*Neanthes virens*	Polychaete
Lumbriculida			
	Lumbriculidae		
		Lumbriculus variegatus	Polychaete
		Insecta	
Diptera			
	Athericidae	*Atherix variegata*	Short-horned flies
	Chironomidae		
		Chironomus plumosus	Midge
		Chironomus tentans	Midge
		Paratanytarsus dissimilis	Midge
		Paratanytarsus parthenogeneticus	Midge
Odonata			
	Coenagrionidae		
		Ischnura verticalis	Eastern forktail
Plecoptera			
	Perlidae	*Claassenia sabulosa*	Stonefly
	Pteronarcyidae		
		Pteronarcella badia	Stonefly
		Pteronarcys californica	Stonefly
		Crustacea	
Diplostraca			
	Daphniidae	*Ceriodaphnia dubia*	Daphnid
		Daphnia magna	Daphnid
		Daphnia pulex	Daphnid
		Simocephalus serrulatus	Daphnid
Podocopida			
	Cyprididae	*Cypris subglobosa*	Ostracod
Amphipoda			
	Crangonyctidae		
		Crangonyx pseudogracilis	Amphipod

	Gammaridae	*Gammarus fasciatus*	Amphipod
		Gammarus lacustris	Amphipod
		Gammarus pseudolimnaeus	Amphipod
	Hyalellidae	*Allorchestes compressa*	Amphipod
		Hyalella azteca	Amphipod
Decapoda			
	Cambaridae	*Orconectes nais*	Crayfish
	Penaeidae	*Farfantepenaeus duorarum*	Pink shrimp
		Metapenaeus dobsoni	Kadal shrimp
Isopoda			
	Asellidae	*Asellus aquaticus*	Isopod
		Caecidotea brevicauda	Isopod
		Caecidotea intermedia	Isopod
Mysida			
	Mysidae	*Americamysis bahia*	Mysid

Echinodermata

Forcipulatida			
	Asteriidae	*Asterias forbesi*	Starfish

Mollusca

Ostreoida			
	Ostreidae	*Crassostrea virginica*	Eastern oyster
Basommatophora			
	Planorbidae	*Planorbella trivolvis*	Snail

Vertebrates

Pisces

Acipenseriformes			
	Acipenseridae		
		Acipenser brevirostrum	Shortnose sturgeon
Atheriniformes			
	Atherinopsidae		
		Menidia beryllina	Inland silverside
		Menidia menidia	Atlantic silverside
Cypriniformes			
	Catastomidae		
		Catostomus commersonii	White sucker
		Xyrauchen texanus	Razorback sucker
	Cyprinidae	*Carassius auratus*	Goldfish
		Cyprinus carpio	Common carp
		Erimonax monachus	Spotfin chub
		Gila elegans	Bonytail chub
		Notropis mekistocholas	Cape fear shiner
		Pimephales promelas	Fathead minnow

		Ptychocheilus lucius	Colorado pikeminnow
Cypriniodontiformes			
	Cyprinodontidae		
		Cyprinodon bovinus	Leon springs pupfish
		Cyprinodon variegatus	Sheepshead minnow
	Poeciliidae	*Gambusia affinis*	Mosquitofish
		Poecilia reticulata	Guppy
		Poeciliopsis occidentalis	Gila topminnow
Esociformes			
	Esocidae	*Esox lucius*	Northern pikeminnow
Gasterosteiformes			
	Gasterosteidae		
		Gasterosteus aculeatus	Threespine stickleback
Mugiliformes			
	Mugilidae	*Chelon labrosus*	Thicklip mullet
Perciformes			
	Centrarchidae		
		Lepomis cyanellus	Green sunfish
		Lepomis macrochirus	Bluegill
		Lepomis microlophus	Redear sunfish
		Micropterus dolomieu	Smallmouth bass
		Micropterus salmoides	Largemouth bass
		Pomoxis nigromaculatus	Black crappie
	Channidae	*Channa marulius*	Bullseye snakehead
	Cichlidae	*Oreochromis mossambicus*	Mozambique tilapia
	Percidae	*Etheostoma fonticola*	Fountain darter
		Etheostoma lepidum	Greenthroat darter
		Perca flavescens	Yellow perch
		Sander vitreus	Walleye
	Sparidae	*Lagodon rhomboides*	Pinfish
Salmoniformes			
	Salmonidae		
		Oncorhynchus clarkii	Cutthroat trout
		Oncorhynchus gilae	Apache trout
		Oncorhynchus kisutch	Coho salmon
		Oncorhynchus mykiss	Rainbow trout
		Oncorhynchus tshawytscha	Chinook salmon
		Salmo salar	Atlantic salmon
		Salmo trutta	Brown trout
		Salvelinus confluentus	Bull trout
		Salvelinus fontinalis	Brook trout
		Salvelinus namaycush	Lake trout
Siluriformes			
	Ictaluridae		
		Ameiurus melas	Black bullhead
		Ictalurus punctatus	Channel catfish

Amphibia

Anura			
	Bufonidae	*Bufo boreas*	Western toad
	Ranidae	*Rana sphenocephala*	Southern leopard frog

III. List of Species in Wildlife Database

The following species were used to develop Web-ICE wildlife species or family-level models.

Aves

Anseriformes			
	Anatidae	*Anas discors*	Bluewinged teal
		Anas domestica	Peking duck
		Anas platyrhynchos	Mallard
		Anas superciliosa	Pacific black duck
		Anas sp.	Pintail
		Anas sp.	Widgeon
		Branta canadensis	Canada goose
		Dendrocygna bicolor	Fulvous whistling duck
Columbiformes			
	Columbidae	*Columba livia*	Rock dove
		Columba oenas	Stock dove
		Columbina inca	Inca dove
		Columbina passerina	Common ground-dove
		Geopelia cuneata	Diamond dove
		Geopelia humeralis	Bar-shouldered dove
		Leptotila verreauxi	White-fronted dove
		Streptopelia risoria	Ringed turtledove
		Streptopelia senegalensis	Laughing dove
		Zenaida asiatica	White-winged dove
		Zenaida auriculata	Eared dove
		Zenaida macroura	Mourning dove
Falconiformes			
	Accipitridae	*Aquila chrysaetos*	Golden eagle
	Falconidae	*Falco sparverius*	American kestrel
Galliformes			
	Odontophoridae		
		Callipepla californica	California quail
		Callipepla gambelii	Gambel’s quail
		Colinus virginianus	Northern bobwhite
	Phasianidae	*Alectoris chukar*	Chukar
		Alectoris rufa	Red partridge
		Centrocercus urophasianus	Sage grouse
		Coturnix japonica	Japanese quail
		Gallus gallus	Chicken
		Meleagris gallopavo	Turkey
		Perdix perdix	Gray partridge
		Phasianus colchicus	Ring-necked pheasant
		Tympanuchus phasianellus	Sharp-tailed grouse
Gruiformes			
	Gruidae	*Grus canadensis*	Sandhill crane

Passeriformes			
	Corvidae	*Aphelocoma* sp.	Scrub jay
		Corcorax melanorhamphos	White-winged chough
		Corvus bennetti	Little Crow
		Corvus brachyrhynchos	American crow
		Corvus corax	Common raven
		Corvus coronoides	Australian raven
		Corvus frugilegus	Rook
		Corvus mellori	Little raven
		Cyanocorax yncas	Green jay
		Pica hudsonia	Black-billed magpie
		Pica nuttalli	Yellowbilled magpie
	Emberizidae	*Junco hyemalis*	Darkeyed junco
		Spizella pallida	Clay-colored sparrow
		Volatinia jacarina	Blue back grassquit
		Zonotrichia atricapilla	Golden-crowned sparrow
		Zonotrichia leucophrys	White-crowned sparrow
	Fringillidae	*Carpodacus mexicanus*	House finch
		Serinus sp.	Canary
	Icteridae	*Agelaius phoeniceus*	Red-winged blackbird
		Agelaius tricolor	Tricolored blackbird
		Euphagus cyanocephalus	Brewer's blackbird
		Molothrus aeneus	Bronzed cowbird
		Molothrus ater	Brown-headed cowbird
		Quiscalus major	Boat-tailed grackle
		Quiscalus quiscula	Common grackle
		Xanthocephalus xanthocephalus	Yellow headed blackbird
	Passeridae	*Neochmia temporalis*	Red-browed firetail
		Passer domesticus	House sparrow
		Passer luteus	Golden sparrow
		Taeniopygia guttata	Zebra finch
	Ploceidae	*Euplectes orix*	Red bishop
		Ploceus cucullatus	Village weaver
		Ploceus taeniopterus	Northern masked weaver
		Quelea quelea	Red billed quelea
	Sturnidae	*Sturnus vulgaris*	Starling
	Turdidae	*Turdus migratorius*	American robin
Psittaciformes			
	Psittacidae	*Aratinga canicularis*	Orange fronted conure
		Aratinga pertinax	Brown-throated conure
		Calyptorhynchus funereus	Yellow tailed black cockatoo
		Melopsittacus undulatus	Budgerigar
		Myiopsitta monachus	Monk parakeet
		Platycercus elegans	Crimson rosella
		Platycercus eximius	Eastern rosella
		Psephotus haematonotus	Red-rumped parrot
Strigiformes			

Strigidae	*Megascops asio*	Eastern screech owl

Mammalia

Artiodactyla		
Bovidae	*Capra hircus*	Domestic goat
	Ovis aries	Domestic sheep
Cervidae	*Odocoileus hemionus*	Mule deer
Carnivora		
Canidae	*Canis familiaris*	Dog
	Canis latrans	Coyote
Lagomorpha		
Leporidae	*Lepus californicus*	Blacktailed jackrabbit
	Oryctolagus cuniculus	Rabbit
Rodentia		
Caviidae	*Caviars porcellus*	Guinea pig
Echimyidae	*Myocastor coypus*	Nutria
Muridae	*Gerbillus* sp.	Gerbil
	Microtus californicus	Meadow mouse
	Microtus pinetorum	Pine mouse
	Microtus sp.	Vole
	Miscrotus pennsylvanicus	Meadow vole
	Mus musculus	Mouse
	Oryzomys palustris	Rice rat
	Peromyscus maniculatus	Deer mouse
	Rattus argentiventer	Ricefield rat
	Rattus exulans	Polynesian rat
	Rattus norvegicus	Norway rat
	Rattus rattus	Roof rat
	Sigmodon hispidus	Cotton rat
Sciuridae	*Cynomys ludovicianus*	Blacktailed prairie dog
	Spermophilus beecheyi	California ground squirrel
	Spermophilus lateralis	Goldenmantled ground squirrel
	Spermophilus richardsonii	Richardsons ground squirrel