

# Web-based Interspecies Correlation Estimation (Web-ICE) for Acute Toxicity: User Manual Version 2.0



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## Abstract

Predictive toxicological models are integral to environmental risk assessment where 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 taxa of interest and that of a surrogate species. Web-ICE includes a total 2081 models for aquatic taxa and 852 models for wildlife taxa. For aquatic species within the same order, Web-ICE models predict within 5-fold and 10-fold of the actual value with 90% and 95% 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 decreases with increasing taxonomic distance.

# 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) software package (version 1.0) 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) expands the fundamental ground work of the original ICE program (Asfaw et al. 2003) as an internet application to include additional chemical and species toxicity data, providing an increased number of interspecies correlations.

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;  $\mu\text{g/L}$ ) and median lethal oral doses for wildlife species (LD50;  $\text{mg/kg}$  bodyweight). ICE models can be used to estimate acute toxicity when a toxicity value for a specific chemical is available for a selected surrogate species or can be estimated (e.g., QSAR), and there is an existing ICE model between the 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 5<sup>th</sup> 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.

# Methods

## I. Database Development

### *Aquatic (Fish and Invertebrates)*

The aquatic database is in development and is currently composed of 4706 LC50/EC50 values for 217 species and 695 chemicals. The data were compiled from the published peer reviewed literature and databases compiled by the U.S. Geological Survey (Mayer and Ellersieck 1986) and the US EPA, including Mayer (1987), ECOTOX/AQUIRE (US EPA 2006), and the Office of Pesticide Programs (OPP) registrant database. All confidential data were censored before inclusion in the public domain database.

Data were used only for tests that adhered to standard acute toxicity test condition requirements of the American Society for Testing and Materials (ASTM 2002, and earlier editions). Data were standardized to similar test conditions and organism life stage to reduce variability. Selection criteria for aquatic test data were as follows:

- 96-hr LC50 data for fish and most invertebrates;
- 96-hr EC50 for most molluscs
- 48-hr EC50 data for daphnids;
- Technical chemicals or formulations  $\geq$  90% active ingredient; and
- Water Quality in accordance with ASTM standards (ASTM 2002).

Open-ended toxicity values (i.e.  $> 100 \mu\text{g/L}$  or  $< 100 \mu\text{g/L}$ ), toxicity values for larval fish and shrimp, adult ("mature") fish, oysters, shrimp, and blue crabs, and duplicate records among multiple sources were not included in model development. When there was more than one toxicity value 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 metals (e.g. copper) and pentachlorophenol were adjusted to 50 mg/L hardness and pH 6.8, respectively (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 I).

### *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 II), which is 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  $\text{Log}_{10}(\text{predicted toxicity}) = a + b \cdot \text{Log}_{10}(\text{surrogate toxicity})$ , 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 an aquatic taxa from a wildlife species, or vice versa.

Only models that had a significant relationship ( $p\text{-value} \leq 0.05$ ) 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: 1074 models comparing 105 species to 105 species;
- 2) Aquatic genera: 481 models comparing 96 species to 33 genera;
- 3) Aquatic family: 526 models comparing 97 species to 32 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 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, interlaboratory 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.

For wildlife species, cross-validation of models showed predicted toxicity values within 5-fold and 10-fold of the actual values with 85% and 95% certainty, respectively. There was a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty increasing with larger taxonomic distance (Raimondo et al., 2007). 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 primary component of Web-ICE (**Web-ICE Modules**) 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). A secondary component, the **Species Sensitivity Distribution Module** is available for aquatic and wildlife species (Figure 1). 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.

The screenshot shows the home page of the Web-ICE program. The page has a blue navigation bar on the left side with the following links: [Exposure Assessment Models](#), [Web-ICE Home](#), [Aquatic Species](#), [Aquatic Genus](#), [Aquatic Family](#), [Wildlife Species](#), [Wildlife Family](#), [Species Sensitivity Distributions](#), [Aquatic](#), [Wildlife](#), [Basic Information](#), [User Manual](#), [Download Model Data](#), and [Bibliography](#). The main content area has a header with the U.S. Environmental Protection Agency logo and the title "Interspecies Correlation Estimation". Below the header is a search bar with a "Go" button. The search bar has a dropdown menu with "All EPA" and "This Area" options. Below the search bar is a breadcrumb trail: "You are here: [EPA Home](#) » [Exposure Assessment](#) » [Food Chain](#) » Web-ICE". The main content area features a large graphic with the text "Web ICE" and a description of the program: "The Web-based Interspecies Correlation Estimation (Web-ICE) application estimates acute toxicity to aquatic and terrestrial organisms for use in risk assessment. Please refer to the [User Manual](#) for detailed instructions on using Web-ICE". Below the graphic is a table titled "Web-ICE Modules" with two columns: "ICE Aquatic" and "ICE Wildlife". The "ICE Aquatic" column lists "Aquatic vertebrates / invertebrates" and links to "Species", "Genus", and "Family". The "ICE Wildlife" column lists "Terrestrial Birds / Mammals" and links to "Species" and "Family". Below the table is a section titled "Species Sensitivity Distribution Module" with links to "ICE Aquatic" and "ICE Wildlife". At the bottom of the page is a footer with contact information: "Please address all comments and questions to the [webmaster](#), [Office of Research and Development](#), [National Health and Environmental Effects Research Laboratory](#), [Gulf Ecology Division](#)".

Figure 1. Home page of Web-ICE program

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

### 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 maybe sorted by surrogate species or predicted taxa to identify available models.


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**Wildlife Species - Taxa Selection Page**



Surrogate:  Predicted:

Sort By:

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 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 (dots), 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, either select the **BACK** button on the browser or select the link to the desired module in the blue navigation bar on left side of the page.

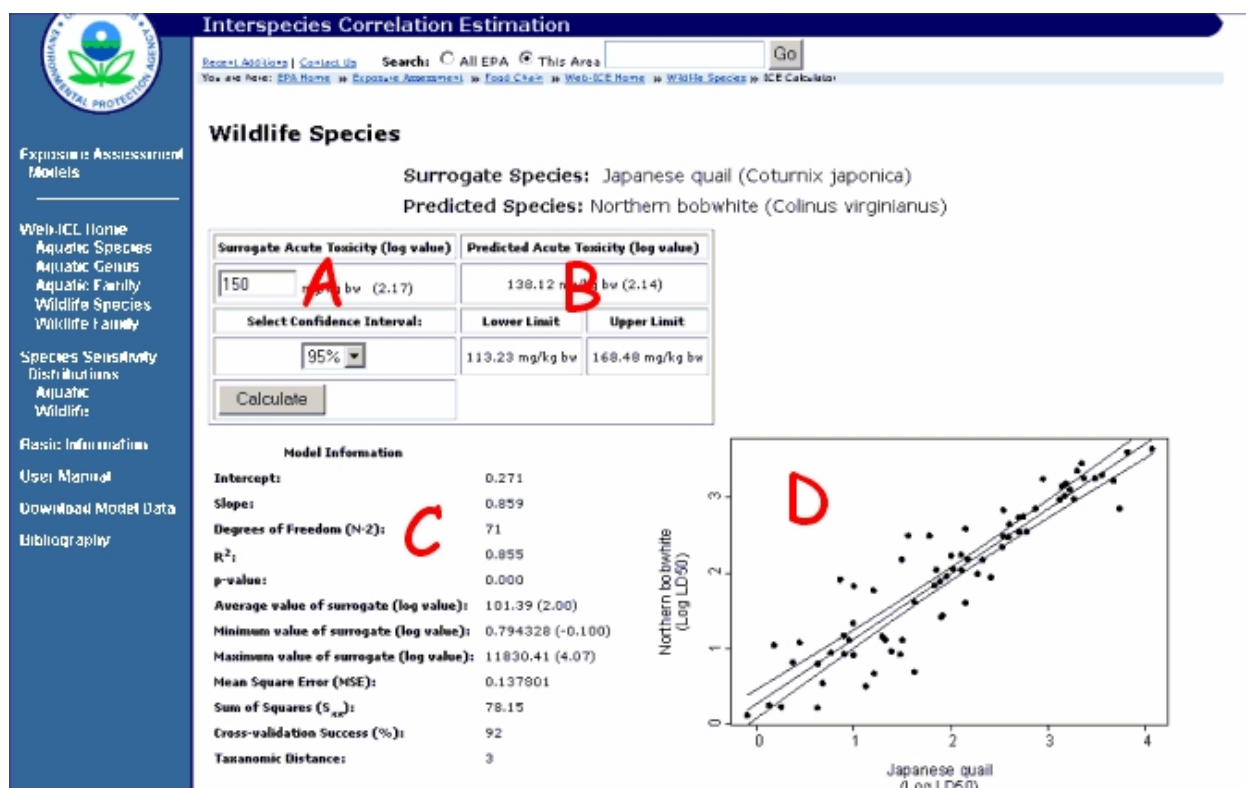


Figure 3. Calculator Page

## 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<sup>th</sup>) of the distribution (Dyer et al. 2006).

The SSD modules for aquatic and wildlife species generate SSDs from Web-ICE estimated toxicity values one or more known toxicity values for 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 multiple 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.

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<sup>st</sup> percentile, **HC5/HD5** corresponds to the 5<sup>th</sup> percentile, and **HC10/HD10** corresponds to the 10<sup>th</sup> 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<sub>10</sub>-transformed environmental concentration (or dose) of the evaluated chemical is represented by C, the parameter,  $\alpha$ , is the sample mean of the log<sub>10</sub>-transformed toxicity values and  $\beta$  is defined as  $\sqrt{3/\pi} * \sigma$ , where  $\sigma$  is the standard deviation of the log<sub>10</sub>-transformed toxicity values (de Zwart 2002). The HC/HD level is determined as the percentile of interest (e.g. 5<sup>th</sup>) 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<sup>th</sup> 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<sup>th</sup> percentile of the SSD developed from the estimated upper limit of each predicted toxicity value.

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**Species Sensitivity Distributions - Aquatic Species**

**Multiple Surrogate SSD**

Surrogate:  Add

Sort By: Common Name

Species	Toxicity	
Blue crab ( <i>Callinectes sapidus</i> )	150	Remove Species
Channel catfish ( <i>Ictalurus punctatus</i> )	200	Remove Species
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	250	Remove Species

Calculate SSD

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Last updated on Tuesday, December 2nd, 2008.  
[file:///L:/Lab/SP2/Task%202%20-%20ICE%20-%20ACE/Web%20ICE/WebICE%20v2.0/iceSSDSpecies.htm?filename=as](#)  
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Figure 4. SSD taxa selection page.

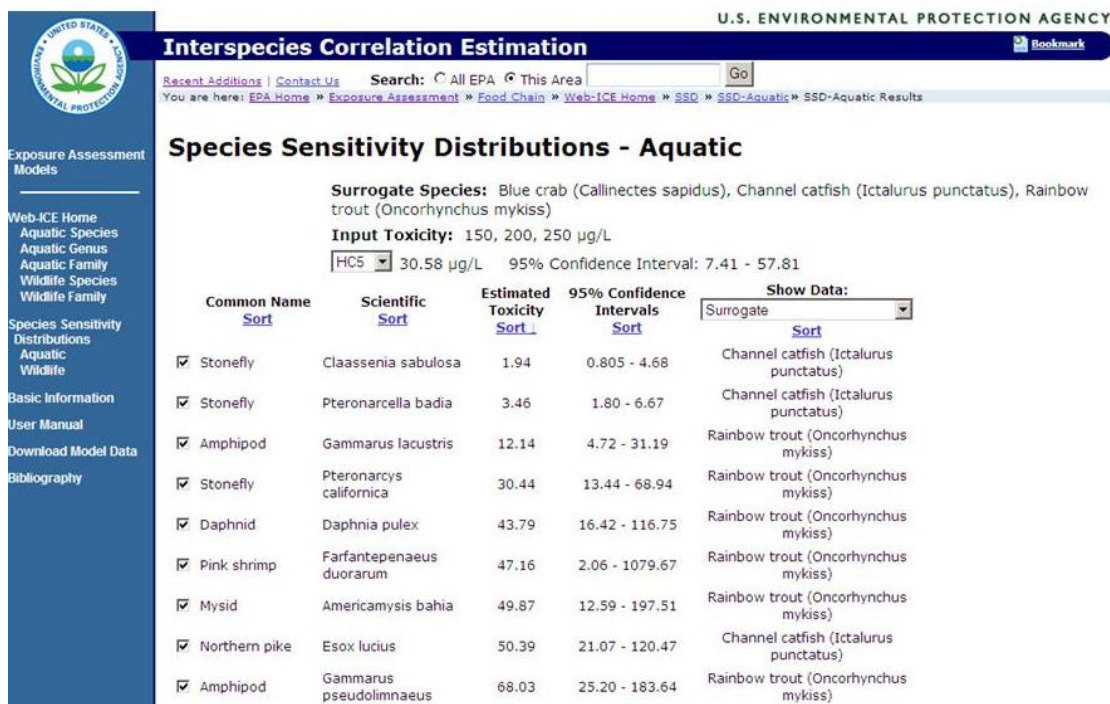


Figure 5. SSD output page.

### 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 maybe changed from 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. Accessing Model Data

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. The raw data used to develop the ICE models is not available due to proprietary rights of some information. A list of chemicals in the aquatic and wildlife databases with the number of species present for each chemical is available for download using the **Chemicals in Aquatic** and **Chemicals in Wildlife** links.

Using model data provided, users may calculate toxicity as:

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

And confidence intervals as:

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

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)** - Reflects the number of data points used to build the model. Degrees of freedom are related to statistical power; in general, the higher the degrees of freedom, the more robust the model.

**R<sup>2</sup>** - The proportion of the data variability that is explained by the model. The greater the R<sup>2</sup> 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** - Describes 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.

## 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. high  $R^2$  value ( $> 0.6$ )
2. low p-values ( $< 0.01$ )
3. high degrees of freedom ( $df > 8$ ,  $N > 10$ )
4. close taxonomic distance ( $\leq 3$ )
5. high cross-validation success rate ( $> 85\%$ )
6. Relatively low mean square error (MSE) ( $< 0.22$ )
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 are recommended to reduce model uncertainty. A priori power analysis determined that linear models with  $df > 8$  have enough statistical power ( $1-\beta > 0.8$ ) to sufficiently increase the chance of finding 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 toxicity estimation.

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 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 ( $\sim 0.13$ ), 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 good 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 maybe 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** dropdown 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 (e.g., endangered species) or a larger number of taxa (species, genera, family) with known uncertainty. Potential applications of Web-ICE generated acute toxicity values 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 intended to reduce the reliance on 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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# Appendix

## I. List of Species in Aquatic Database

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

### Invertebrates

#### *Annelida*

Haplotaxida		
Tubificidae	<i>Varichaetadrilus pacificus</i>	Oligochaete
Scolecida		
Capitellidae	<i>Capitella capitata</i>	Polychaete
Aciculata		
Nereididae	<i>Neanthes arenaceodentata</i>	Polychaete
	<i>Neanthes virens</i>	Polychaete

#### *Insecta*

Diptera		
Athericidae	<i>Atherix variegata</i>	Short-horned flies
Chironomidae	<i>Chironomus plumosus</i>	Midge
	<i>Paratanytarsus dissimilis</i>	Midge
Plecoptera		
Perlidae	<i>Claassenia sabulosa</i>	Stonefly
Pteronarcyidae	<i>Pteronarcella badia</i>	Stonefly
	<i>Pteronarcella californica</i>	Stonefly

#### *Crustacea*

Amphipoda		
Gammaridae	<i>Gammarus fasciatus</i>	Amphipod
	<i>Gammarus lacustris</i>	Amphipod
	<i>Gammarus pseudolimnaeus</i>	Amphipod
Hyalellidae	<i>Hyalella azteca</i>	Amphipod
Isopoda		
Asellidae	<i>Caecidotea brevicauda</i>	Isopod
Diplostraca		
Daphniidae	<i>Ceriodaphnia reticulata</i>	Daphnid
	<i>Daphnia magna</i>	Daphnid
	<i>Daphnia pulex</i>	Daphnid
	<i>Simocephalus serrulatus</i>	Daphnid
	<i>Simocephalus vetulus</i>	Daphnid
Decapoda		
Cambaridae	<i>Orconectes nais</i>	Crayfish
Canceridae	<i>Cancer magister</i>	Dungeness crab

Nephropidae	<i>Homarus americanus</i>	American lobster
Ocypodidae	<i>Uca pigulator</i>	Fiddler crab
Palaemonidae	<i>Palaemonetes kadiakensis</i>	Mississippi grass shrimp
	<i>Palaemonetes pugio</i>	Daggerblade grass shrimp
Penaeidae	<i>Farfantepenaeus duorarum</i>	Pink shrimp
	<i>Litopenaeus setiferus</i>	White shrimp
Portunidae	<i>Callinectes sapidus</i>	Blue crab
	<i>Carcinus maenas</i>	Green crab
Mysida		
Mysidae	<i>Americamysis bahia</i>	Mysid
Calanoida		
Acartiidae	<i>Acartia clausi</i>	Copepod
	<i>Acartia tonsa</i>	Copepod
Temoridae	<i>Eurytemora affinis</i>	Copepod
<b>Mollusca</b>		
Myioda		
Myidae	<i>Mya arenaria</i>	Softshell clam
Mytiloida		
Mytilidae	<i>Mytilus edulis</i>	Blue mussel
Ostreoida		
Ostreidae	<i>Crassostrea virginica</i>	Eastern oyster
Pectinidae	<i>Argopecten irradians</i>	Bay scallop
Unionoida		
Unionidae	<i>Actinonaias pectorosa</i>	Pheasantshell
	<i>Lampsilis straminea</i>	Southern fatmucket
	<i>Lampsilis teres</i>	Yellow sandshell
	<i>Utterbackia imbecillis</i>	Paper pondshell
	<i>Villosa iris</i>	Rainbow mussel
	<i>Villosa vibex</i>	Southern rainbow
	<i>Villosa villosa</i>	Downy rainbow
Veneroida		
Mactridae	<i>Rangia cuneata</i>	Atlantic rangia
Basommatophora		
Physidae	<i>Aplexa hypnorum</i>	Snail
	<i>Physella gyrina</i>	Tadpole physa snail
Neogastropoda		
Nassariidae	<i>Nassarius obsoletus</i>	Eastern mud snail
<b>Miscellaneous</b>		
Forcipulatida		
Asteroiidae	<i>Asterias forbesi</i>	Starfish
Plumatellida		
Lophopodidae	<i>Lophopodella carteri</i>	Bryozoan
Pectinatellidae	<i>Pectinatella magnifica</i>	Bryozoan

## Vertebrates

## *Pisces*

Acipenseriformes		
Acipenseridae	<i>Acipenser brevirostrum</i>	Shortnose sturgeon
Anguilliformes		
Anguillidae	<i>Anguilla rostrata</i>	American eel
Atheriniformes		
Atherinopsidae	<i>Leuresthes tenuis</i> <i>Menidia beryllina</i> <i>Menidia menidia</i> <i>Menidia peninsulae</i>	California grunion Inland silverside Atlantic silverside Tidewater silverside
Cypriniformes		
Catastomidae	<i>Catostomus commersonii</i> <i>Xyrauchen texanus</i>	White sucker Razorback sucker
Cyprinidae	<i>Carassius auratus</i> <i>Cyprinus carpio</i> <i>Erimonax monachus</i> <i>Gila elegans</i> <i>Notropis mekistocholas</i> <i>Pimephales promelas</i> <i>Ptychocheilus lucius</i> <i>Ptychocheilus oregonensis</i>	Goldfish Common carp Spotfin chub Bonytail chub Cape fear shiner Fathead minnow Colorado pikeminnow Northern pikeminnow
Cyprinodontiformes		
Cyprinodontidae	<i>Cyprinodon bovinus</i> <i>Cyprinodon variegatus</i> <i>Jordanella floridae</i>	Leon springs pupfish Sheepshead minnow Flagfish
Fundulidae	<i>Fundulus diaphanus</i> <i>Fundulus heteroclitus</i>	Banded killifish Mummichog
Poeciliidae	<i>Gambusia affinis</i> <i>Poecilia reticulata</i> <i>Poeciliopsis occidentalis</i>	Mosquitofish Guppy Gila topminnow
Esociformes		
Esocidae	<i>Esox lucius</i>	Northern pikeminnow
Mugiliformes		
Mugilidae	<i>Mugil cephalus</i>	Striped mullet
Perciformes		
Centrarchidae	<i>Lepomis cyanellus</i> <i>Lepomis gibbosus</i> <i>Lepomis macrochirus</i> <i>Lepomis microlophus</i> <i>Micropterus dolomieu</i> <i>Micropterus salmoides</i> <i>Pomoxis nigromaculatus</i>	Green sunfish Pumpkinseed sunfish Bluegill Redear sunfish Smallmouth bass Largemouth bass Black crappie
Cichlidae	<i>Oreochromis mossambicus</i>	Mozambique tilapia
Moronidae	<i>Morone americana</i> <i>Morone saxatilis</i>	White perch Striped bass
Percidae	<i>Etheostoma fonticola</i>	Fountain darter



	<i>Etheostoma lepidum</i>	Greenthroat darter
	<i>Perca flavescens</i>	Yellow perch
	<i>Sander vitreus</i>	Walleye
Sciaenidae	<i>Leiostomus xanthurus</i>	Spot
Salmoniformes		
Salmonidae	<i>Oncorhynchus clarkii</i>	Cutthroat trout
	<i>Oncorhynchus gilae</i>	Apache trout
	<i>Oncorhynchus kisutch</i>	Coho salmon
	<i>Oncorhynchus mykiss</i>	Rainbow trout
	<i>Oncorhynchus tshawytscha</i>	Chinook salmon
	<i>Salmo salar</i>	Atlantic salmon
	<i>Salmo trutta</i>	Brown trout
	<i>Salvelinus fontinalis</i>	Brook trout
	<i>Salvelinus namaycush</i>	Lake trout
Siluriformes		
Ictaluridae	<i>Ameiurus melas</i>	Black bullhead
	<i>Ictalurus punctatus</i>	Channel catfish
	<b><i>Amphibia</i></b>	
Anura		
Bufonidae	<i>Bufo boreas</i>	Western toad
	<i>Bufo fowleri</i>	Fowlers toad
Hylidae	<i>Pseudacris triseriata</i>	Western chorus frog
Ranidae	<i>Rana catesbeiana</i>	Bullfrog
	<i>Rana pipiens</i>	Northern leopard frog
	<i>Rana sphenoccephala</i>	Southern leopard frog

## II. List of Species in Wildlife Database

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

### *Aves*

#### Anseriformes

Anatidae	<i>Anas discors</i>	Bluewinged teal
	<i>Anas domestica</i>	Peking duck
	<i>Anas platyrhynchos</i>	Mallard
	<i>Anas superciliosa</i>	Pacific black duck
	<i>Anas</i> sp.	Pintail
	<i>Anas</i> sp.	Widgeon
	<i>Branta canadensis</i>	Canada goose
	<i>Dendrocygna bicolor</i>	Fulvous whistling duck

#### Columbiformes

Columbidae	<i>Columba livia</i>	Rock dove
	<i>Columba oenas</i>	Stock dove
	<i>Columbina inca</i>	Inca dove
	<i>Columbina passerina</i>	Common ground-dove
	<i>Geopelia cuneata</i>	Diamond dove
	<i>Geopelia humeralis</i>	Bar-shouldered dove
	<i>Leptotila verreauxi</i>	White-fronted dove
	<i>Streptopelia risoria</i>	Ringed turtledove
	<i>Streptopelia senegalensis</i>	Laughing dove
	<i>Zenaida asiatica</i>	White-winged dove
	<i>Zenaida auriculata</i>	Eared dove
	<i>Zenaida macroura</i>	Mourning dove

#### Falconiformes

Accipitridae	<i>Aquila chrysaetos</i>	Golden eagle
Falconidae	<i>Falco sparverius</i>	American kestrel

#### Galliformes

Odontophoridae	<i>Callipepla californica</i>	California quail
	<i>Callipepla gambelii</i>	Gambel's quail
	<i>Colinus virginianus</i>	Northern bobwhite
Phasianidae	<i>Alectoris chukar</i>	Chukar
	<i>Alectoris rufa</i>	Red partridge
	<i>Centrocercus urophasianus</i>	Sage grouse
	<i>Coturnix japonica</i>	Japanese quail
	<i>Gallus gallus</i>	Chicken
	<i>Meleagris gallopavo</i>	Turkey
	<i>Perdix perdix</i>	Gray partridge
	<i>Phasianus colchicus</i>	Ring-necked pheasant
	<i>Tympanuchus phasianellus</i>	Sharp-tailed grouse

#### Gruiformes

Gruidae	<i>Grus canadensis</i>	Sandhill crane
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Passeriformes

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

Strigiformes

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