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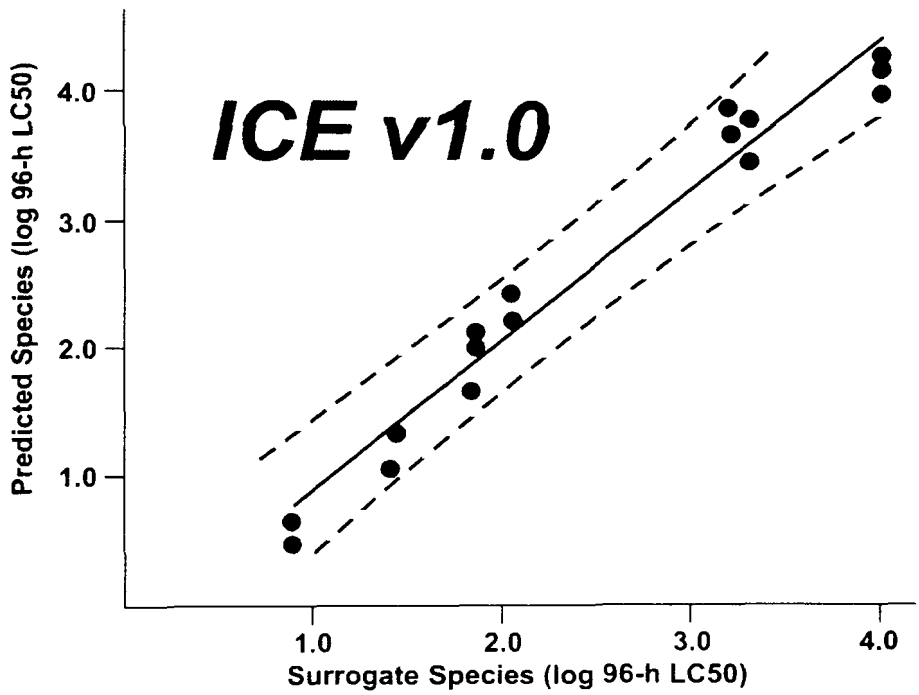
II. User Manual and Software



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Interspecies Correlation Estimations (ICE) for Acute Toxicity To Aquatic Organisms and Wildlife

II. User Manual and Software

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Notice

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Abstract

Predictive toxicological models, including estimates of uncertainty, are necessary to address probability-based ecological risk assessments. A method and software (ICE) were developed for estimating acute toxicity of chemicals to species, genera, and families when data are lacking. Interspecies correlation models for acute toxicity (4082 models) were derived for 143 aquatic and terrestrial organisms using Model II least squares regression, where both variables are independent and subject to measurement error ($\log X_2 = a + b\,[\log X_1]$). Toxicity of a chemical to one species can be predicted from toxicity to another species with known certainty. Correlations are generally best within a taxonomic family, decreasing with increasing taxonomic distance. However, certain species (e.g., rainbow trout) were found to be the most useful of all species for acute estimations among taxa, including families. Correlations for wildlife species were not as good, in general, as those for aquatic species, but routes of exposure are different - - oral or dietary versus respiratory, respectively.

Introduction

Acute and chronic toxicity testing of several species is required for protection of the numerous species represented in environmental habitats. Realistically, the number of species tested is limited by test procedure, species availability, time and expense. Thus, environmental managers must frequently perform risk analyses and make decisions regarding chemicals, mixtures, and effluents for which even acute data are minimal or do not exist. This is of particular concern for the protection of endangered species that are unavailable to test, other species that have not been tested or are not feasible to test, and when minimal data sets exist for a chemical.

To address this problem, interspecies correlations with selected organisms were conducted relating acute toxicity of a chemical for one species that of another (Mayer et al. 1987, 2004). The approach integrates species sensitivity to chemicals with species taxonomic similarities (physiology, biochemistry) using correlation methodology. This allows for estimation of acute toxicity of a chemical to many species from toxicity values of only one or a few species. However, application of this methodology is extremely time consuming without automation and software.

This software program, Interspecies Correlation Estimation (ICE), described herein, allows the user to estimate acute toxicity for a species or higher taxa (genus, family) having no acute toxicity data from a species having acute data. ICE will, therefore, greatly enhance the use of probability-based risk assessments for chemicals having minimal data sets and will extend the utility of quantitative structure-activity relationships QSAR (Lipnick 1995), from one species (i.e., fathead minnow, *Pimephales promelas*) to many species. Also, if an acute toxicity test is to be conducted, ICE can be used to more accurately identify the range of exposure concentrations required. ICE is based on the Windows platform and is specifically designed for estimating acute toxicity to aquatic and terrestrial organisms and providing graphical and tabular presentation of results.

Data Base

Three data sets (aquatic, wildlife, wildlife subacute) were used for correlation analyses. The aquatic data set was a compilation of Mayer (1987), Mayer and Ellersieck (1986), ECOTOX (U.S. Environmental Protection Agency 2002), and the U.S. Environmental Protection Agency's Office of Pesticide Programs (OPP) aquatic data (247 species, 661 chemicals, 4778 tests). Data used were based mainly on tests conducted with technical-grade chemicals, and water temperature, pH, hardness, and salinity generally conforming to requirements of ASTM (2002). Nominal or measured concentrations (µg/L) were based on active ingredients (≥90%), with the exception of metal salts, which were based on metal content. The data set was standardized additionally by using only static tests. The chemicals represent all major pesticides, as well as numerous industrial and inorganic chemicals. The aquatic data set was used to compare species, species versus genera, and species versus families. Two wildlife sets, single oral dose or *per os* (47 species, 316 chemicals, 893 tests) and 5-day dietary (19 species, 214 chemicals, 493 tests) were analyzed. The two data sets consisted of data from: 1) acute *per os* tests with data (mg chemical/kg of body weight) from Hudson et al. (1984), 2) 5-day dietary subacute tests with data (mg chemical/kg dry weight of diet) from Hill et al. (1975), and 3) the OPP data base of both *per os* and 5-day dietary tests. Wildlife data sets, mainly birds and mammals, were partially standardized by using only tests with technical-grade chemicals. No correlations could be derived for wildlife species versus genera. Detailed descriptions of the data sets can be found in Buckler et al. (2003) and Mayer et al. (2004).

Software Language

The ICE software is based on a Windows platform and written in Visual Basic (Microsoft Visual Basic 2000). Subroutines (Fortran programs) in Visual Basic are required to call Fortran IMSL routines necessary in certain calculations (Compaq Fortran, Visual Numeric 1999). **See Software Development** and **Interpretation of Statistics** for detailed methodology.

Installing ICE

System Requirements

- Operates on Microsoft® Windows 95, 98, 2000, NT and XP (Windows® 98 or later is suggested).
- Minimum 16MB RAM (64 MB or greater is suggested).
- CPU speed of over 200 MHz is suggested; ICE will work with less, but is very slow acquiring equations.
- 6MB hard disk space.
- Mouse or pointing device.
- Printer (optional).

Remove any existing versions of ICE before installing the new one or malfunctions may occur.

To remove old ICE software:

1. Double click **My Computer.**
2. Double click **Control Panel.**
3. Double click **Add/Remove Programs.**
4. Click **ICE.**
5. Click **Delete** or **Change/Remove.**
6. Install new ICE software.

To install new ICE software:

1. Place the ICE CD in the CD ROM drive.
2. Click **Start** button.
3. Select **Run** from the menu.
4. Select **Browse** from the **Run** window.
5. Select the drive letter associated with the CD drive from the Browse window (or ICE July 17 2003) [D:]).
6. Double-click **Setup** or **D:\Setup.EXE** file.
7 Click **OK.**
8. Windows now walks you through the installation process.
9. Following installation, the ICE program can be accessed by clicking **Start, Programs**, and then **ICE**. You can create an icon on the Desktop screen by placing the mouse pointer on the ICE icon, holding down the control button, and dragging the icon to desired location on the screen.

Using ICE in Windows

To start the program, double click the ICE icon and select a surrogate species for which you have an acute value (ICE window 1, Fig. 1). Select data sets in **Options** (See Options and Model sections for available data sets). Enter the toxicity value in μg/L for aquaticspecies (mg chemical/kg of body weight for wildlifespecies and wildlifefamily; mg chemical/kg dry weight of

diet for wildlifesubacute) where the value of 100 (default value) is the X_1 row; press Enter. After a surrogate species is chosen, a second list of species or taxa (X_2) will appear for which you can select and estimate the acute toxicity value (ICE window 2, Fig. 2). Also at this time, the logo will disappear and be replaced with a line graph and confidence limits. Click on an X_2 taxa to estimate its acute toxicity value; additional X_2 taxa may be selected from this window. Click on the **Back** command located in the upper left corner to select another surrogate (X_1) species; this will produce a different listing of X_2 species or taxa. After choosing the X_1 and X_2 species, the program lists statistics and a graph; as you go from one X_2 species to another, difference statistics and graphics are produced for that particular model.

Model Selection

The following is recommended to provide the best confidence in the estimates made with the ICE program:

1. Use equations for species within the same genus or family.
2. Use equations that have degrees of freedom (df) ≥ 3 ($n \geq 5$).
3. Use equations that have a significant ($p \leq 0.05$) correlation (slope, *b*).
4. If data for more than one potential surrogate species (X_1) exists:
   a. If n for surrogate$_1$ = n for surrogate$_2$, use equation with the highest *r* value.
   b. If n for surrogate$_1$ ≠ n for surrogate$_2$, use equation with smallest error mean square (EMS).
5. If equations for species do not exist in aquaticspecies or wildlifespecies, search for its genus or family in aquaticgenus, aquaticfamily, or wildlifefamily. Generally, species within a genus or family will have more similar sensitivities to the same chemicals than more distantly related taxa.

ICE Application Windows

When first opened, the program will appear with the ICE logo to the right and a list of surrogate species (X_1) in the upper left box (Fig. 1). Scroll down to find the surrogate species of interest and click on it. The screen will automatically go to ICE window 2 (Fig. 2); see following numbers for explanation.

1. List of species (X_2) or taxa for which acute toxicity values can be estimated from a known surrogate species value (X_1). Click on X_2 species or taxa of interest. The X_2 species list changes depending on which surrogate species is chosen. If a specific surrogate and X_2 species or taxa have three or more chemicals in common, then a regression equation will be presented.

2. Level of statistical Type 1 Error (α) used to determine specifice t values (e.g., 1%, 5%, etc.) and confidence bands and may be changed in the **Options** window by user.

3. $\boldsymbol{X_1}$ is the acute toxicity value associated with the surrogate species under the column Actual. The number to the right (under the column **Log-Base 10**) is the same number, but the log base 10 ($\log_{10}$) of that number. The number 100 (default value) will first appear under the **Actual** column. To change the 100 value to the acute value of the surrogate species, click on the light green box and enter the surrogate species acute value (µg/L for aquatic species [aquaticspecies, aguaticgenus, aquaticfamily]; mg chemical/kg body weight [wildlifespecies, wildlifefamily] or mg chemical/kg dry weight of diet [wildlifesubacute] for wildlife).

4. $\boldsymbol{X_2}$ is the estimated acute value for species or taxa

5. **Upper** and **Lower** confidence limits for the estimated acute toxicity value – Note the two sets of confidence limits listed; one not associated with **P** (pooled) and one associated with **P**. The confidence limits not associated with **P** represent the confidence limits for that particular

Figure 1. ICE window 1

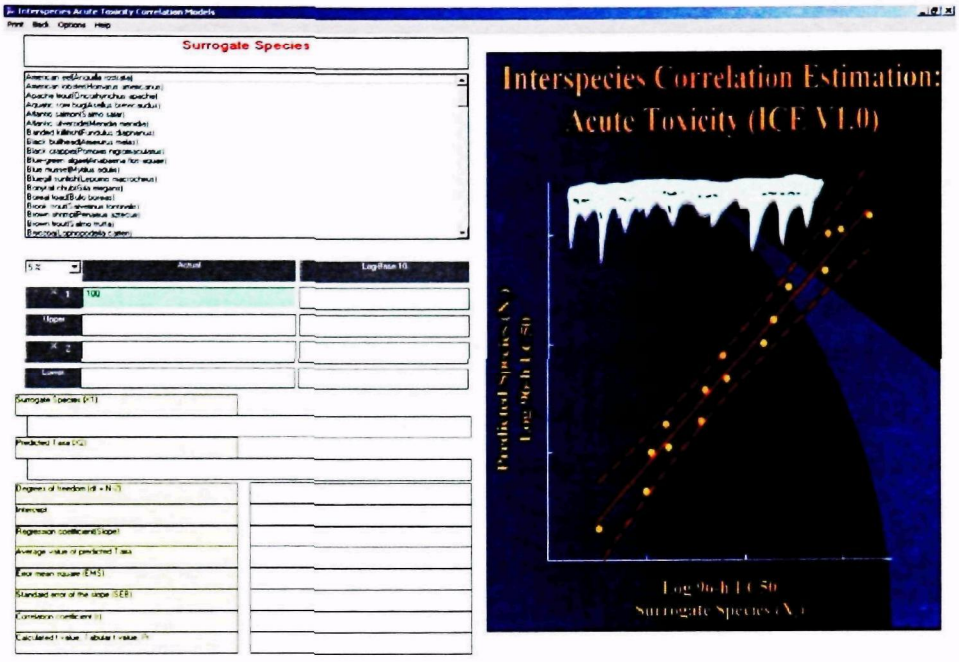
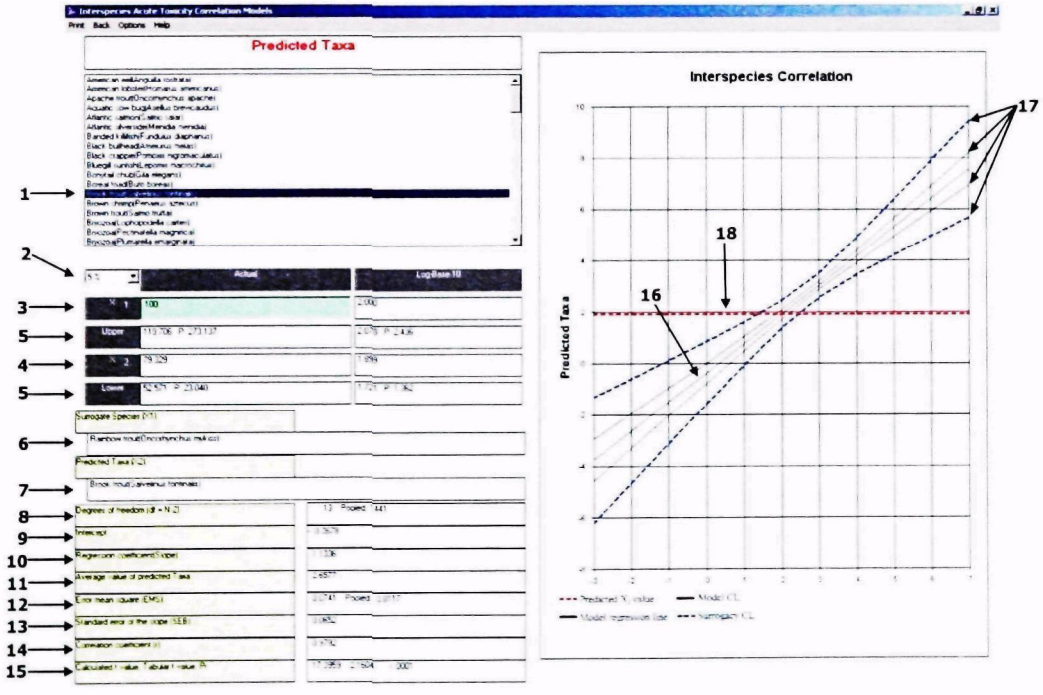


Figure 2. ICE window 2



species-species (or taxa) equation (uncertainty due to model). The confidence limits associated with **P** represent a pooled variance for that surrogate species with all other species equations (uncertainty due to surrogacy).

6. **Surrogate Species** (X_1) is name of the selected surrogate species.
7. **Predicted Taxa** (X_2) is name of the species or taxa for which acute toxicity is being estimated.
8. **Degrees of freedom** (**df = n – 2**) associated with each equation. The first **df** is based on the number of chemicals that X_1 and X_2 have in common. The second **df** (Pooled) represents the sum of df for that specific surrogate species and its equations among all other species.
9. **Intercept** (*a*) is the $\log_{10}$ EC/LC/LD50 for the X_2 species or taxa when the $\log_{10}$ value for the surrogate species (X_2) is equal to 0.
10. **Regression coefficient** (**Slope**) or *b* represents the $\log_{10}$ change in X_2 for every 1.0 $\log_{10}$ change in X_1.
11. **Average value of predicted taxa** is the average acute toxicity value for X_2 species or taxa based on df + 2 (or n).
12. **Error mean square** (**EMS**) represents the variance associated with the regression line. The **Pooled** value represents the sum of the error sum of squares associated with each equation divided by the pooled df.
13. **Standard error of slope** (**SEB**) is the standard error of the regression coefficient (slope or *b*).
14. **Correlation coefficient** (*r*) is the mutual linear association between X_1 and X_2 species or taxa.
15. This window contains two t values (**Calculated t value, Tabular t value**) and the actual level of significance (**Pr**). The **Calculated t value** is a calculated t statistic to test the significance of the relationship between X_1 and X_2. It is calculated by dividing the slope by the standard error of the slope (calculated t = *b*/SEB). The **Tabular t value** is a two-tailed tabulated t value from a standard t table. If the **Calculated t value** is ≥ **Tabular t value**, a significant relationship exists between the X_1 and X_2 species (i.e., regression line is significantly different from 0). A specific α level may be selected by changing the % for a Type 1 error rate (see 2 above) or the actual level of significance (**Pr**) may be used.
16. Graphic representation of the regression line from the statistics (see 9-13 above), with all values expressed as $\log_{10}$.
17. Curved lines represent confidence bands based on values for X_2 species or taxa. Two sets of confidence bands exist: solid and broken lines. The solid lines are derived for that specific species to species/genus/family equation (uncertainty due to model) and broken lines represent uncertainty due to surrogacy (see 5 and 12 above).
18. The horizontal line identifies the estimated $\log_{10}$ acute toxicity value where it crosses the X_2 axis.

Menu Bar

The menu bar (Fig. 3) contains four commands: **Print, Back, Options,** and **Help**.

Figure 3. Menu bar

- **Print** – To print, click **Print**. There are two options: 1) click **Single** and the present screen is printed, or 2) click **All** and all equations associated with the surrogate species and the selected data set are printed. Printing is accomplished on the default printer. If the printer supports zooming, the screen will be enlarged or reduced to fit in a landscape orientation. An alternative method of printing is to copy the screen displayed by simultaneously pressing ALT and Print Screen on the keyboard. This output can then be pasted to another program such as Microsoft® Word or Power Point, then printed from one of those programs.
- **Back** – Returns to ICE window 1 to select another surrogate species.
- **Options** – Allows setting program options (Fig. 4). The first option is choosing a data set.
- **Data Sets (Common names)** offers selection of data sets with species common name first followed by scientific name and **Data Sets (Scientific names)** provides the same data sets with species scientific name first followed by the common name. Click on the data set desired (Fig. 5), then **Open** (bottom right of window), followed by **Select** in the Options window. You can now work with the data set in the ICE program. The default data set is aquatic species versus a variety of aquatic species (aquaticspecies). As described in the following Graphics section, the captions for the graph can be changed in the Options window. At the bottom center of the Options window is the significance level (α) in % for confidence bands and t tests; it can be changed here or at item 2 on the main screen. The **Select** command will save all changes, and **Cancel** will only eliminate changes made while in the Options window. Changes made at the end of a session will remain when the ICE program is started again. Click **Default** to return everything back to the default settings.
- **Help** – A narrative of the documentation. It is outlined according to major subjects. Print documentation by clicking on the subject and then clicking **print**.

Graphics

Double click on the graph to fill the screen; double click again to return to the original size. The graph can be manipulated by clicking on the **Options** command (upper left). Click on the appropriate box in the Options window (Fig. 4) and type in desired caption changes for the X_1 and X_2 axes and the title. Then click on the **Select** command to install the changes. If the new captions do not fit on the graph, click on each caption and drag to fit the allowable space. Click **Default** to return all altered captions on the graph back to default settings. To exit the Options screen, click the upper right **X** on the Options window.

Exit

To exit the ICE program, click the **X** in the upper right corner of the screen.

Figure 4. Options window

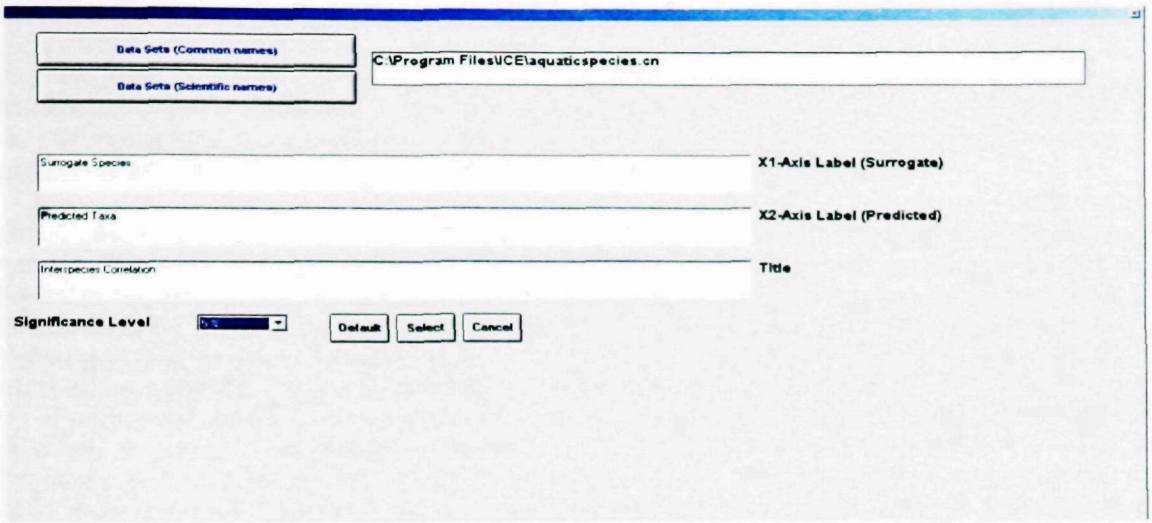
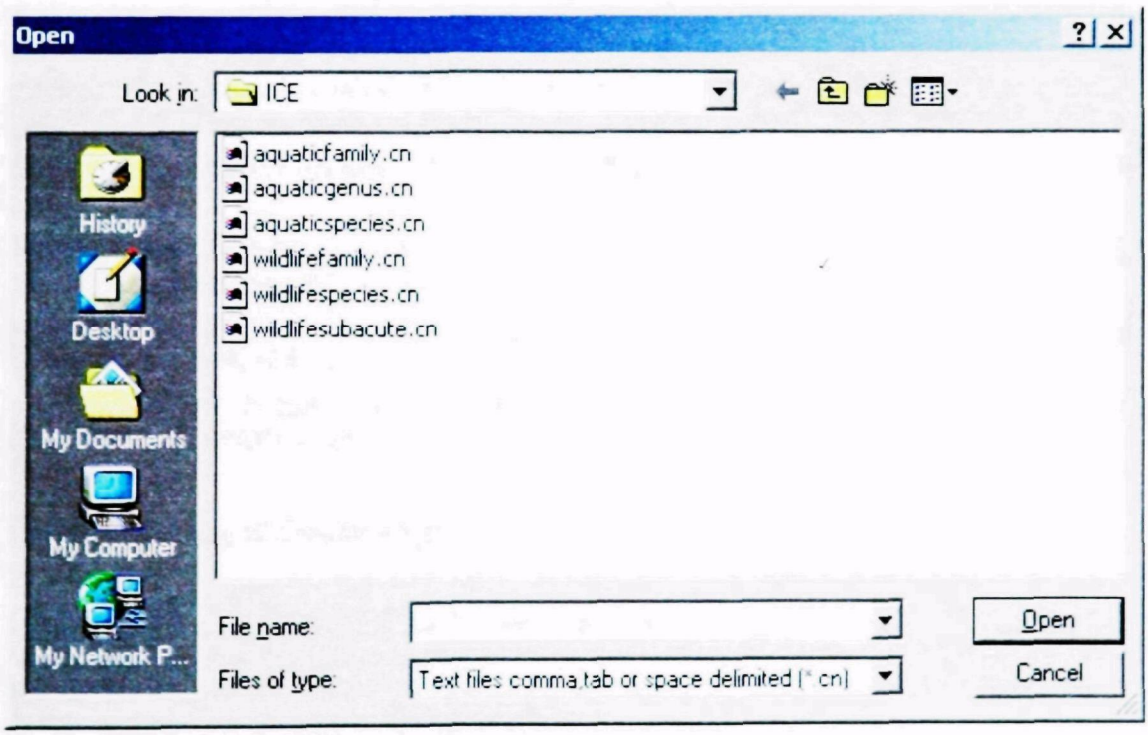


Figure 5. Data sets window



Software Development

Model

Interspecies correlations ($Y = a + bX$) were conducted using Model II least squares methodology (Snedecor and Cochran 1980) where both variables are random (both variables are independent and subject to measurement error). Different notations are used for model $Y = a + bX$ (i.e., $X_2 = a + bX_1$), because intertaxa toxicity comparisons are true correlations. For that reason, the correlation coefficient r, a measure of the mutual linear association between two variables (X_1 and X_2), is used instead of the coefficient of determination r^2 (the proportion of the variability of the dependent variable Y that is caused or explained by the independent variable X).

Slopes (b), intercepts (a), and other statistics were derived from the equation $\log X_2 = a + b(\log X_1)$, where X_1 equals the acute toxicity value for a surrogate species and X_2 equals the acute toxicity value for another species (or genus or family). Species with paired tests on three or more chemicals were the minimum requirement for inclusion in each analysis, although five or more are recommended (Mayer and Ellersieck 1986). When either of the paired species included more than one acute value (EC, LC, or LD50), the geometric mean was used (Fig. 6). For genus and family, a surrogate species was compared to all genera and families having acute geometric mean values for two or more individual species. These individual values were used for analyses (i.e., a genus or family geometric mean was not used, Fig. 7). The surrogate species was not included in its own genus or family when those comparisons were made. A rough estimate of surrogate species/genus or surrogate species/family can be made with aquaticspecies, with the understanding that you are using only one species to represent a genus or family. In summary, six equation data sets exist for ICE with aquaticspecies being the default data set for the software program; they are:

1. aquaticspecies – Aquatic species; 2914 models; 119 species versus 119 species; EC or LC50 in µg/L
2. aquaticgenus – Aquatic species; 371 models; 96 species versus 14 genera; EC or LC50 in µg/L
3. aquaticfamily – Aquatic species; 490 models; 102 species versus 13 families; EC or LC50 in µg/L
4. wildlifespecies – Wildlife species; 278 models; 25 species versus 25 species; LD50 in mg chemical/kg of body weight
5. wildlifefamily – Wildlife species; 61 models; 23 species versus 5 families; LD50 in mg chemical/kg of body weight
6. wildlifesubacute – Wildlife species; 14 models; 6 species versus 6 species; LC50 in mg chemical/kg dry weight of diet

Statistical Analyses and Equation Formation Procedures

All equations were generated using SAS (1999). An algorithm was written to pair every species with every other species (or genus or family) by common chemical. PROC GLM was then used to calculate the regression equation of $\log_{10}$ predicted taxa = $a + b^*\log_{10}$ surrogate species where $a = X_2$ intercept and b = regression coefficient (slope).

Another computer procedure was written to capture the necessary statistics to generate the equation for the data sets above. This is the same procedure to be used when data sets not

Figure 6. Species versus species correlation.

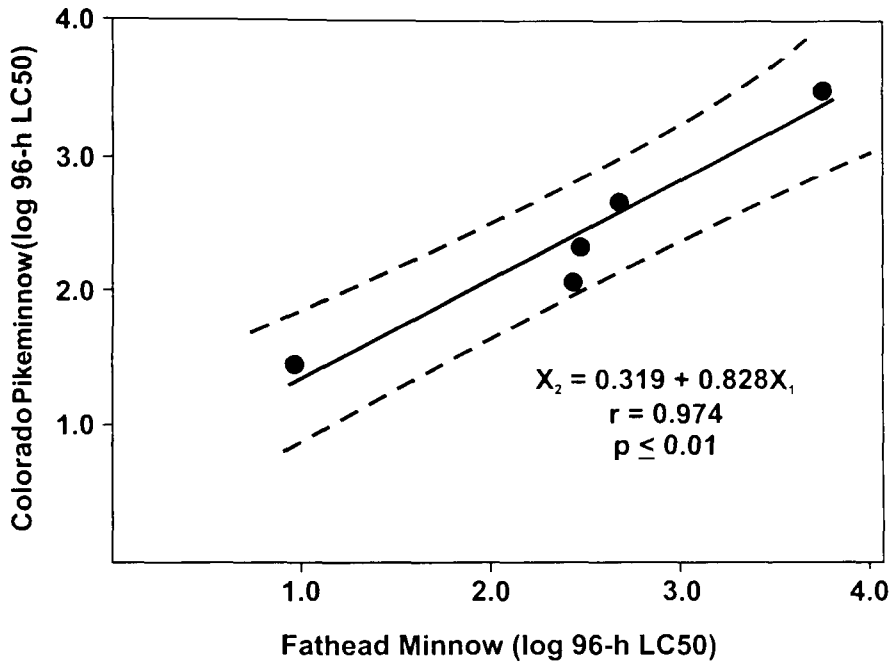
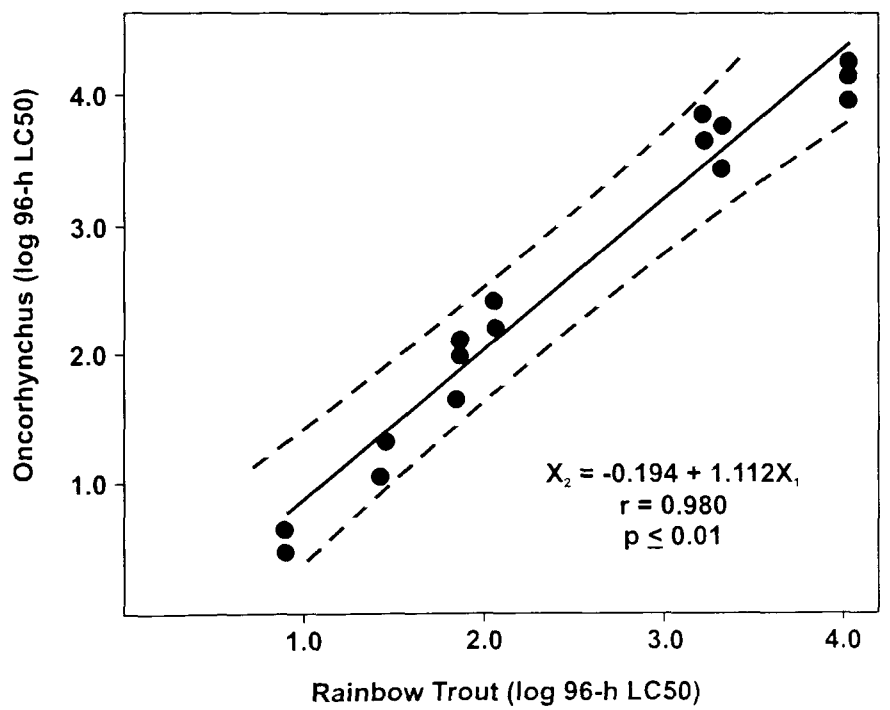


Figure 7. Species versus genus correlation.



included in ICE are of interest. In order for ICE to be able to read data, the procedure performs the following functions:

1. From the SAS output, capture the following parameters: surrogate species (X_1) name, predicted species or taxa (X_2) name, sample size, intercept, regression coefficient (slope), predicted species mean, error mean square, standard error of the slope, correlation coefficient (r), and probability that slope is significant.
2. Enter the parameters in Microsoft® Excel and save them as comma, tab or space delimited files. If space delimited files are used, a data value can not contain spaces. For this reason comma or tab delimited files are preferred.
3. If more than one equation is calculated, sort the file by X_1 and X_2.

All equation data sets can be viewed in Microsoft® Excel or other spreadsheet software that can read ASCII text files. All files supplied are comma delimited. The order of the parameters is as follows, which each letter representing a column in the spreadsheet.

A = Surrogate species (X_1)
B = Predicted taxa (X_2)
C = Sample size (n) for which each equation is based (df = n - 2)
D = Intercept (a)
E = Regression coefficient (slope b)
F = Average value of the predicted taxa
G = Error mean square (EMS)
H = Standard error of the regression coefficient (SEB)
I = Correlation coefficient (r)
J = Probability (Pr) that the slope is not equal to 0

Interpretation of Statistics

The purpose of the ICE software is to estimate an acute toxicity value for an untested species or taxa ($\boldsymbol{X_2}$) from an actual test value for the surrogate species ($\boldsymbol{X_1}$). If a model for the $\boldsymbol{X_2}$ species does not exist, an estimate may be made by using higher taxonomic levels (genus or family) containing that $\boldsymbol{X_2}$ species. The accuracy of the estimated value can be judged visually by the closeness of the confidence bands to the regression line. The closer the confidence bands are to the estimated value, the higher the confidence in the estimate. In certain cases, where the correlation may be less than acceptable, the confidence in accuracy may be enhanced by the correlative strength of the surrogate species selected. This occurs when the confidence bands for uncertainty due to surrogacy is smaller than the uncertainty due to the specific model.

ICE provides a number of other statistics that estimate the accuracy of prediction. The first statistic to evaluate is the significance of the correlation between $\boldsymbol{X_1}$ and $\boldsymbol{X_2}$ or when slope (b) $\neq 0$ (see 15, Fig. 2). This is accomplished by a t test and comparing the **Calculated t value** to the **Tabular t value** or by using the actual significance level (**Pr**). If the **Calculated t value** is equal to or greater than the **Tabular t value**, then the correlation is significant at the α level selected. The **Pr** value should be ≤ 0.05 for the correlation (slope, b) to be significant. When the regression coefficient (slope b) is close to 1.0, chemicals affect the $\boldsymbol{X_1}$ and $\boldsymbol{X_2}$ species in a similar fashion. The **Intercept** (a) can be used to determine if chemicals are generally more or less toxic to one species or taxa than another: negative intercept, the $\boldsymbol{X_2}$ species or taxa are generally more sensitive; positive intercept, the $\boldsymbol{X_1}$ species are generally more sensitive.

The next statistic to assess is the **Correlation coefficient** (*r*). The larger the ***r*** value and the closer it is to 1.0, the stronger the acute toxicity relationship is between the two taxa selected. However, *r* can sometimes be misleading in that it can be very high, but the t-test statistic may not show a significant relationship. This most frequently occurs when the degrees of freedom (df) are low and/or the slope is close to zero. We recommend that the degrees of freedom be at least 3 (or n $\geq$ 5) in order to increase confidence in the equations (Mayer and Ellersieck 1986). However, all equations having degrees of freedom of $\geq$ 1 (n $\geq$ 3) were included, because many species do not have existing or acceptable data available. These equations are intended to show the relationship, based on the available data. For further information on calculation and interpretation of linear regression analysis, see Ellersieck and LaPoint (1995).

Be aware that the prediction of sensitivity of one species from another by a regression equation is not the same equation if reversed. This is why two different equations are needed. The only time an equation can predict in either direction is if $r^2 = 1.0$. The following is a proof to demonstrate.

Let:
$\bar{y}$ = average value of Y
$\bar{x}$ = average value of X
$\hat{y}$ = predicted value Y
$\hat{x}$ = predicted value of X
Y_c = *substitute value of* Y

The linear regression of Y on X with slope b:

$$\hat{y} = \bar{y} + b(x - \bar{x})$$

$$\hat{y} = (\bar{y} - b\bar{x}) + bx \quad (\hat{y} = \text{intercept} + \text{slope [x]})$$

Substitute a particular $Y = Y_c$ *for* $\hat{y}$ and solve for $\hat{x}$.

This is the predicted X when $Y = Y_c$

$$\hat{x} = (Y_c - \bar{y} + b\bar{x})/b = (Y_c - \bar{y})/b + \bar{x} \qquad (1)$$

The linear regression of X on Y with slope g:

$$\hat{x} = (\bar{x} \quad g\bar{y}) + gY \quad (\hat{x} = \textit{intercept} + \textit{slope}\,[Y])$$
$$\hat{x} = (\bar{x} - g\bar{y}) + gY_c$$

Substitute g from $r = \sqrt{bg} \Rightarrow g = r^2/b$ in the above equation.

$$\hat{x} = (\bar{x} - r^2\bar{y}/b) + r^2 Y_c/b = (Y_c - \bar{y})r^2/b + \bar{x} \qquad (2)$$

Compare equation (1) and (2); the two will be equal only when $r^2 = 1$.

If there is no variance around the regression line, the error mean square will equal zero; thus, all points will fit exactly on the regression line. This is the only time that $r^2 = 1$.

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Quick Reference

1. **Installing ICE**
   - Insert disk into CD ROM drive
   - Click **Start** button.
   - Select **Run** from menu.
   - Select **Browse** from the **Run** window.
   - Select drive letter associated with CD Drive from the **Browse** window (or **ICE July 17, 2003 [D:]**).
   - Double Click **Setup** or **D:\SETUP.EXE** file.
   - Click **OK**.
   - Windows now walks you through installation process.
   - Following installation, click **Start, Programs**, and then **ICE**; drag ICE icon to desired screen location.

2. **Using ICE**
   - Open ICE program.
   - Select data set in **Options** (**Data Sets**, select data set, **Open**, then **Select**); aquaticspecies = aquatic surrogate species vs. estimated species (aquaticgenus, species vs. genus; aquaticfamily, species vs. family), wildlifespecies = wildlife surrogate species vs. estimated species (wildlifefamily, species vs. family; per os), wildlifesubacute = wildlife surrogate species vs. estimated species (dietary).
   - Select Surrogate Species (X_1) having an acute toxicity value.
   - Enter surrogate species acute toxicity value at the 100 default value (μg/L for aquaticspecies, aquaticgenus, aquaticfamily; mg/kg body weight for wildlifespecies and wildlifefamily; mg/kg diet for wildlifesubacute).
   - Select Predicted Taxa (X_2) to estimate acute toxicity.
   - Select **Back** in menu bar to choose another surrogate species; select **Options** to choose another data set.
   - Select **Print** then **Single** to print that frame or **All** to print all correlations for the chosen surrogate species within that data set.

3. **Choosing best correlations**
   - Use equations for surrogate and predicted taxa within same genus or family.
   - Use equations having df ≥ 3 ($n \geq 5$).
   - Use equations having a significant ($p \leq 0.05$) correlation (slope, *b*).
   - If data for more than one surrogate exists:
     $n_1 = n_2$, use equation having highest *r* value
     $n_1 \neq n_2$, use equation having smallest error mean square value.
   - If equations for the species to be estimated do not exist in aquaticspecies or wildlifespecies, search for its genus or family in aquaticgenus, aquaticfamily, or wildlifefamily.