**Summary**

 SSDs were fit to predicted test results for fish exposed to Malathion. Five distributions were tested against four sets of predicted values. The first set was predicted from rainbow trout (*Onchorhynchus mykiss*) data, the second from bluegill (*Lepomis macrochirus*) data, the third from fathead minnow data (*Pimephales promelas*), and the fourth was predicted from a mixture of all three surrogates. Only data for species typically tested in freshwater were used.

Summary statistics for SSDs are presented below. Note that the statistics presented in the table below, with the exception of noting the best distribution for each species, are model-averaged estimates from across all fitted distributions.

Table 1. Summary statistics for SSDs fit to Malathion test results

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| statistic | rainbow trout | bluegill | fatheadminnow | mixture | empirical |
| Best Distribution (by AICc) | logistic | triangular | logistic | triangular | normal |
| CV of the HC05 | 0.15 | 0.26 | 0.22 | 0.26 | 0.51 |
| HC05 | 43.1 | 26.0 | 1645.7 | 23.9 | 44.8 |
| HC10 | 49.9 | 32.6 | 2090.0 | 32.7 | 89.7 |
| HC50 | 93.4 | 79.6 | 5235.7 | 137.9 | 1364.9 |
| HC90 | 225.2 | 213.0 | 13728.8 | 1315.6 | 29214.6 |
| HC95 | 309.8 | 288.1 | 18199.3 | 3112.4 | 80293.0 |
| Mortality Thresh.1 (slope = 4.5) | 3.8 | 2.3 | 144.6 | 2.1 | 3.9 |
| Indirect Effects Threshold1 (slope = 4.5) | 22.4 | 13.5 | 854.2 | 12.4 | 23.3 |

1Slope of dose-response curve = 4.5

Predicted values from rainbow trout, bluegill, and the mixture of all three surrogates produced estimates of the HC05 that were lower than, but comparable to the empirical data (Table 1). However, predicted values from fathead minnow produced estimates of the HC05 that were almost two orders of magnitude greater than the empirical value. HC05 estimates from the predicted data appeared to be more precisely estimated (smaller coefficients of variation and smaller estimates of sampling variance in the HC05). Plotted SSDs for the mixed data set show severe lack of fit, and this is confirmed in many significant P-values for lack of fit in the individual model tables below. For each fitted distribution, I also calculated where the upper confidence limit of the HC05 fell on the fitted distribution and this typically ranged from the tenth to the 25th quantile. Though there are no hard guidelines, values above the 20th percentile perhaps should be viewed with some suspicion, as indicating substantial uncertainty in the HC05 relative to the variance of the distribution. Predicted value SSDs had smaller variances than the SSD generated from empirical data. When the surrogate species rainbow trout and bluegill are examined on the empirical dataset (Fig. 5) they are seen to be among the more sensitive species. In contrast, the other surrogate species (fathead minnow) is among the more tolerant species (Fig. 5). Thus, even with a much smaller variance, the estimated HC05 comes out close to that estimated using the empirical data for bluegill and rainbow trout, but not for fathead minnow.

 Crosstabulation of predicted values with empirical test results show that Web ICE predictions are significantly positively correlated with empirically measured toxicity values (correlation coefficients range from 0.72 – 0.80). However, for all three surrogates, the discrepancy between predicted and measured toxicity values increases with dissimilarity between the surrogate species used to derive the predicted values and the predicted species. For species with quite dissimilar tolerances, the discrepancies between measured and predicted toxicity approached 2 orders of magnitude. This effect may be related to phylogeny. Thus predicted values get worse with taxonomic distance, as the dissimilarity in tolerance grows between two taxa (but perhaps not for predicted values from fathead minnow). This point should be investigated further.

**I. Data**

Data were received from Elizabeth Donovan on 2 October 2014 in the file:

 “Malathion SSD\_WebICE\_10.02.14.xlsx”

From this file I created three working files:

 1. MalathionFWFishIcePredRainbowTrout.xlsx

2. MalathionFWFishIcePredBluegill.xlsx

3. MalathionFWFishIcePredFathead.xlsx

4. MalathionFWFishIcePredAllThree.xlsx

These working files each contain three fields (Table 2).

Table 2. Fields in working files

|  |  |  |
| --- | --- | --- |
| Field in working files | Field in received file | Column in received file |
| 1. Genus | Scientific | C |
| 2. Species | Scientific | C |
| 3. Toxicity | Measured Toxicity (ug/L) | D |

Table 3. Distribution of predicted values available for Malathion

|  |  |
| --- | --- |
| Surrogate Species | Predicted Values |
| rainbow trout | 46 |
| bluegill | 36 |
| fathead minnow | 45 |
| All three | 33 |

For each analysis I also added the surrogate species empirical test value(s) to the dataset to allow comparison of the predicted distribution to the value of the surrogate species. For further comparison, I also reanalyzed the previous empirical dataset on freshwater fish provided by Elizabeth Donovan (“Malathion\_SSD Criteria\_UPDATE.xlsx”, 9 July 2014) after converting that dataset from mg/L to µg/L. Previous analyses of that data were provided in the draft report “Species Sensitivity Distributions for Aquatic Vertebrates Malathion.docx” (16 July 2014).

I considered five potential distributions for the Malathion data (log-normal, log-logistic, log-triangular, log-gumbel, and Burr). To fit each of the first four distributions, I first common log (log10) transformed the toxicity values. I also explored the best-fit distribution using Akaike’s information criterion. Finally I calculated the direct and indirect effect thresholds and report five quantiles from the fitted SSDs (HC05, HC10, HC50, HC90, HC95).

**II. Comparison of distributions using AICc**

I began by using AICc to compare the five distributions for both datasets. For these comparisons all SSDs were fit using maximum likelihood.

Based on AICc the datasets disagreed on which distribution best fit the predicted values. For values predicted from rainbow trout, the gumbel distribution fit best, but was followed closely by the burr distribution (Table 4). For the bluegill and fathead minnow predicted value datasets, the triangular distribution, followed by the normal distribution were selected. For the predicted values from a mixture of all three surrogates, the gumbel and burr distribution were selected as best by AICc, but both showed considerable evidence of lack-of-fit. Finally, for the empirical data, the normal distribution provided the best fit, followed closely by the gumbel and triangular distributions (Table 7).

Table 4. Comparison of distributions for freshwater fish toxicity values predicted from rainbow trout.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| distribution | HC05 | AICc | ∆AICc | Weight |
| gumbel | 45.0 | 310.57 | 0.00 | 0.60 |
| burr | 45.0 | 313.10 | 2.52 | 0.17 |
| triangular | 38.7 | 314.15 | 3.57 | 0.10 |
| normal | 36.7 | 315.09 | 4.52 | 0.06 |
| logistic | 34.2 | 315.16 | 4.59 | 0.06 |

Table 5. Comparison of distributions for freshwater fish toxicity values predicted from bluegill.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| distribution | HC05 | AICc | ∆AICc | Weight |
| triangular | 27.2 | 191.08 | 0.00 | 0.50 |
| normal | 24.0 | 192.83 | 1.75 | 0.21 |
| gumbel | 27.5 | 193.35 | 2.27 | 0.16 |
| logistic | 21.2 | 194.38 | 3.30 | 0.10 |
| burr | 27.5 | 196.34 | 5.26 | 0.04 |

Table 6. Comparison of distributions for freshwater fish toxicity values predicted from fathead minnow.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| distribution | HC05 | AICc | ∆AICc | Weight |
| triangular | 1694.9 | 314.02 | 0.00 | 0.54 |
| normal | 1601.8 | 315.59 | 1.57 | 0.25 |
| logistic | 1495.1 | 316.98 | 2.96 | 0.12 |
| gumbel | 1681.1 | 318.15 | 4.13 | 0.07 |
| burr | 1664.1 | 320.97 | 6.95 | 0.02 |

Table 7. Comparison of distributions for freshwater fish toxicity values predicted from all three surrogates.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| distribution | HC05 | AICc | ∆AICc | Weight |
| gumbel | 23.9 | 466.85 | 0.00 | 0.77 |
| burr | 23.9 | 469.31 | 2.46 | 0.23 |
| logistic | 5.6 | 481.36 | 14.51 | 0.00 |
| triangular | 6.8 | 481.42 | 14.57 | 0.00 |
| normal | 7.2 | 482.52 | 15.67 | 0.00 |

Table 8. Comparison of distributions for empirical freshwater fish toxicity data for Malathion

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| distribution | HC05 | AICc | ∆AICc | Weight |
| normal | 38.9 | 790.71 | 0.00 | 0.44 |
| gumbel | 56.9 | 792.12 | 1.41 | 0.22 |
| triangular | 48.9 | 792.63 | 1.92 | 0.17 |
| logistic | 30.9 | 793.46 | 2.75 | 0.11 |
| burr | 56.9 | 794.44 | 3.73 | 0.07 |

The cumulative distribution functions for each of the above 4 datasets are plotted below.

Figure 1. Model-averaged SSD for WebICE predicted values for freshwater fish LC50s for Malathion, based on rainbow trout regressions.



Figure 2. Model-averaged SSD for WebICE predicted values for freshwater fish LC50s for Malathion, based on bluegill regressions.



Figure 3. Model-averaged SSD for WebICE predicted values for freshwater fish LC50s for Malathion, based on fathead minnow regressions.



Figure 4. Model-averaged SSD for WebICE predicted values for freshwater fish LC50s for Malathion, based on all three surrogates.



Figure 5. Model-averaged SSD for Malathion LC50s for freshwater fish. Red points indicate single toxicity values. Black points indicate multiple toxicity values. Blue line indicates full range of toxicity values for a given taxon.



**IV. Goodness of fit and the importance of fitting method**

To test goodness-of-fit all five distributions were fit to each of the five datasets and bootstrap goodness-of-fit tests with 5,000 bootstrap replicates were run. Three different fitting methods were used (maximum likelihood, moment estimators, and graphical methods), though not all methods are available for all distributions. Tables 9 – 13 give results of these fitting exercises. Significant lack of fit was observed for many distributions fit to the mixture data from all three surrogates. The burr distribution showed significant lack of fit for the empirical data.

Table 9. Range of HC05 values (μg/L) for Malathion SSDs estimated from Web ICE predicted values using rainbow trout

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| distribution | method | HC05 | SE | CV | LCL | UCL | LCQ | UCQ | P |
| gumbel | ML | 44.95 | 4.94 | 0.11 | 37.56 | 56.89 | 0.01 | 0.16 | 1.00 |
| gumbel | MO | 44.56 | 5.98 | 0.13 | 34.03 | 57.49 | 0.01 | 0.17 | 1.00 |
| gumbel | GR | 40.70 | 6.22 | 0.15 | 25.97 | 50.71 | 0.00 | 0.13 | 1.00 |
| normal | ML | 36.70 | 6.79 | 0.18 | 26.41 | 52.84 | 0.01 | 0.15 | 1.00 |
| normal | MO | 36.03 | 6.72 | 0.19 | 25.62 | 52.01 | 0.01 | 0.14 | 1.00 |
| normal | GR | 32.67 | 6.37 | 0.19 | 19.60 | 44.11 | 0.01 | 0.11 | 1.00 |
| logistic | ML | 34.21 | 6.95 | 0.20 | 23.06 | 50.22 | 0.02 | 0.14 | 0.98 |
| logistic | MO | 36.52 | 7.52 | 0.21 | 24.01 | 53.82 | 0.02 | 0.14 | 0.98 |
| logistic | GR | 31.51 | 6.96 | 0.22 | 15.55 | 42.93 | 0.01 | 0.10 | 0.93 |
| triangular | ML | 38.71 | 6.12 | 0.16 | 32.09 | 55.94 | 0.02 | 0.16 | 1.00 |
| triangular | MO | 35.36 | 6.27 | 0.18 | 25.80 | 50.29 | 0.01 | 0.15 | 1.00 |
| triangular | GR | 33.36 | 6.05 | 0.18 | 22.42 | 45.86 | 0.00 | 0.13 | 1.00 |
| burr | ML | 44.95 | 4.96 | 0.11 | 37.11 | 56.61 | 0.01 | 0.16 | 0.42 |

Table 10. Range of HC05 values (μg/L) for Malathion SSDs estimated from Web ICE predicted values using bluegill

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| distribution | method | HC05 | SE | CV | LCL | UCL | LCQ | UCQ | P |
| gumbel | ML | 27.46 | 5.52 | 0.20 | 20.29 | 41.65 | 0.01 | 0.20 | 0.99 |
| gumbel | MO | 29.95 | 6.29 | 0.21 | 19.70 | 44.53 | 0.00 | 0.21 | 1.00 |
| gumbel | GR | 25.66 | 6.29 | 0.25 | 12.06 | 37.05 | 0.00 | 0.17 | 0.93 |
| normal | ML | 23.97 | 7.17 | 0.30 | 14.83 | 43.19 | 0.01 | 0.20 | 1.00 |
| normal | MO | 23.08 | 7.14 | 0.31 | 13.53 | 41.33 | 0.01 | 0.19 | 1.00 |
| normal | GR | 19.39 | 6.37 | 0.33 | 8.04 | 32.45 | 0.00 | 0.15 | 0.97 |
| logistic | ML | 21.22 | 7.64 | 0.36 | 11.42 | 40.82 | 0.01 | 0.18 | 0.86 |
| logistic | MO | 23.46 | 7.91 | 0.34 | 12.24 | 42.92 | 0.01 | 0.18 | 0.90 |
| logistic | GR | 18.19 | 6.51 | 0.36 | 5.51 | 30.44 | 0.01 | 0.13 | 0.71 |
| triangular | ML | 27.17 | 6.65 | 0.24 | 20.90 | 46.43 | 0.01 | 0.21 | 1.00 |
| triangular | MO | 22.55 | 6.70 | 0.30 | 13.79 | 39.44 | 0.00 | 0.19 | 1.00 |
| triangular | GR | 20.17 | 6.22 | 0.31 | 10.05 | 33.80 | 0.00 | 0.16 | 1.00 |
| burr | ML | 27.45 | 5.83 | 0.21 | 18.94 | 41.28 | 0.01 | 0.20 | 0.41 |

Table 11. Range of HC05 values (μg/L) for Malathion SSDs estimated from Web ICE predicted values using fathead minnow

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| distribution | method | HC05 | SE | CV | LCL | UCL | LCQ | UCQ | P |
| gumbel | ML | 1681.13 | 384.20 | 0.23 | 1204.86 | 2702.85 | 0.01 | 0.21 | 1.00 |
| gumbel | MO | 1993.72 | 430.86 | 0.22 | 1314.50 | 3006.58 | 0.00 | 0.22 | 1.00 |
| gumbel | GR | 1699.49 | 428.58 | 0.25 | 778.36 | 2449.15 | 0.00 | 0.17 | 1.00 |
| normal | ML | 1601.82 | 513.66 | 0.32 | 963.24 | 2970.80 | 0.01 | 0.21 | 1.00 |
| normal | MO | 1539.30 | 481.43 | 0.31 | 886.35 | 2746.43 | 0.01 | 0.19 | 1.00 |
| normal | GR | 1285.06 | 422.82 | 0.33 | 518.72 | 2180.06 | 0.00 | 0.15 | 1.00 |
| logistic | ML | 1495.09 | 542.92 | 0.36 | 793.64 | 2868.72 | 0.01 | 0.18 | 1.00 |
| logistic | MO | 1564.73 | 535.68 | 0.34 | 791.21 | 2918.15 | 0.01 | 0.19 | 1.00 |
| logistic | GR | 1202.95 | 438.84 | 0.36 | 344.01 | 2018.11 | 0.00 | 0.13 | 1.00 |
| triangular | ML | 1694.92 | 439.58 | 0.26 | 1304.83 | 3019.07 | 0.01 | 0.23 | 1.00 |
| triangular | MO | 1504.45 | 454.93 | 0.30 | 912.67 | 2667.03 | 0.00 | 0.19 | 1.00 |
| triangular | GR | 1338.79 | 426.94 | 0.32 | 666.12 | 2291.67 | 0.00 | 0.17 | 1.00 |
| burr | ML | 1664.12 | 429.66 | 0.26 | 1135.97 | 2810.55 | 0.01 | 0.22 | 0.18 |

Table 12. Range of HC05 values (μg/L) for Malathion SSDs estimated from Web ICE predicted values using all three surrogates

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| distribution | method | HC05 | SE | CV | LCL | UCL | LCQ | UCQ | P |
| gumbel | ML | 23.92 | 6.36 | 0.27 | 15.92 | 40.41 | 0.01 | 0.14 | 0.35 |
| gumbel | MO | 13.82 | 6.38 | 0.46 | 6.00 | 30.70 | 0.01 | 0.16 | 0.02 |
| gumbel | GR | 10.50 | 4.58 | 0.44 | 2.79 | 20.44 | 0.00 | 0.12 | 0.00 |
| normal | ML | 7.21 | 5.39 | 0.75 | 2.64 | 23.04 | 0.02 | 0.14 | 0.13 |
| normal | MO | 6.83 | 4.82 | 0.71 | 2.27 | 20.36 | 0.01 | 0.13 | 0.11 |
| normal | GR | 5.09 | 3.31 | 0.65 | 1.06 | 13.41 | 0.01 | 0.11 | 0.05 |
| logistic | ML | 5.61 | 3.98 | 0.71 | 1.88 | 16.75 | 0.02 | 0.13 | 0.01 |
| logistic | MO | 7.14 | 5.80 | 0.81 | 1.84 | 24.42 | 0.02 | 0.13 | 0.03 |
| logistic | GR | 4.57 | 3.06 | 0.67 | 0.56 | 11.71 | 0.01 | 0.10 | 0.01 |
| triangular | ML | 6.80 | 4.56 | 0.67 | 3.86 | 21.02 | 0.02 | 0.15 | 0.44 |
| triangular | MO | 6.42 | 4.36 | 0.68 | 2.45 | 18.95 | 0.01 | 0.14 | 0.38 |
| triangular | GR | 5.41 | 3.41 | 0.63 | 1.61 | 14.51 | 0.00 | 0.13 | 0.25 |
| burr | ML | 23.89 | 6.49 | 0.27 | 15.41 | 40.89 | 0.01 | 0.15 | 0.00 |

Table 13. Range of HC05 values for Malathion SSDs based on measured freshwater fish LC50s

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| distribution | method | HC05 | SE | CV | LCL | UCL | LCQ | UCQ | P |
| gumbel | ML | 56.95 | 22.13 | 0.39 | 31.96 | 116.60 | 0.02 | 0.13 | 0.51 |
| gumbel | MO | 77.72 | 32.32 | 0.42 | 35.99 | 159.20 | 0.01 | 0.14 | 0.83 |
| gumbel | GR | 61.19 | 24.03 | 0.39 | 19.77 | 109.86 | 0.00 | 0.11 | 0.52 |
| normal | ML | 38.89 | 25.60 | 0.66 | 15.15 | 112.62 | 0.02 | 0.13 | 0.60 |
| normal | MO | 37.26 | 24.48 | 0.66 | 14.13 | 105.56 | 0.02 | 0.12 | 0.57 |
| normal | GR | 29.05 | 15.69 | 0.54 | 7.37 | 68.04 | 0.01 | 0.10 | 0.41 |
| logistic | ML | 30.93 | 23.60 | 0.76 | 9.87 | 100.16 | 0.02 | 0.12 | 0.19 |
| logistic | MO | 39.04 | 28.51 | 0.73 | 12.11 | 119.54 | 0.02 | 0.12 | 0.30 |
| logistic | GR | 26.54 | 16.20 | 0.61 | 3.91 | 64.43 | 0.01 | 0.09 | 0.14 |
| triangular | ML | 48.94 | 30.96 | 0.63 | 27.39 | 146.47 | 0.02 | 0.13 | 1.00 |
| triangular | MO | 34.91 | 20.61 | 0.59 | 14.18 | 92.93 | 0.01 | 0.13 | 1.00 |
| triangular | GR | 30.38 | 16.10 | 0.53 | 10.24 | 70.86 | 0.01 | 0.11 | 1.00 |
| burr | ML | 56.92 | 21.85 | 0.38 | 30.74 | 114.94 | 0.02 | 0.13 | 0.00 |

**V. Calculation of other quantiles**

Tables 14 - 18 provide estimates of the HC05 as well as other quantiles of the fitted SSDs.

Table 14. Estimated quantiles of the fitted SSDs for Malathion Web ICE predicted LC50s based on rainbow trout.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| dist | method | HC05 | HC10 | HC50 | HC90 | HC95 |
| gumbel | ML | 45.0 | 51.0 | 90.9 | 224.9 | 318.0 |
| gumbel | MO | 44.6 | 50.7 | 91.0 | 228.4 | 324.5 |
| gumbel | GR | 40.7 | 47.1 | 91.9 | 262.2 | 391.4 |
| normal | ML | 36.7 | 45.9 | 100.9 | 221.9 | 277.4 |
| normal | MO | 36.0 | 45.2 | 100.9 | 225.1 | 282.6 |
| normal | GR | 32.7 | 41.9 | 100.9 | 242.9 | 311.6 |
| logistic | ML | 34.2 | 44.2 | 94.1 | 200.1 | 258.6 |
| logistic | MO | 36.5 | 47.3 | 100.9 | 215.4 | 278.8 |
| logistic | GR | 31.5 | 42.3 | 100.9 | 240.5 | 323.1 |
| triangular | ML | 38.7 | 47.1 | 107.4 | 245.2 | 298.1 |
| triangular | MO | 35.4 | 43.2 | 100.9 | 235.5 | 287.9 |
| triangular | GR | 33.4 | 41.2 | 100.9 | 246.9 | 305.2 |
| burr | ML | 45.0 | 51.0 | 90.9 | 224.8 | 317.8 |

Table 15. Estimated quantiles of the fitted SSDs for Malathion Web ICE predicted LC50s based on bluegill.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| dist | method | HC05 | HC10 | HC50 | HC90 | HC95 |
| gumbel | ML | 27.5 | 32.7 | 72.0 | 249.2 | 400.4 |
| gumbel | MO | 30.0 | 35.1 | 72.0 | 222.7 | 342.8 |
| gumbel | GR | 25.7 | 31.0 | 73.3 | 283.1 | 474.4 |
| normal | ML | 24.0 | 31.4 | 81.7 | 212.5 | 278.6 |
| normal | MO | 23.1 | 30.5 | 81.7 | 218.8 | 289.3 |
| normal | GR | 19.4 | 26.6 | 81.7 | 250.6 | 344.3 |
| logistic | ML | 21.2 | 29.8 | 80.6 | 218.1 | 306.0 |
| logistic | MO | 23.5 | 32.2 | 81.7 | 207.3 | 284.5 |
| logistic | GR | 18.2 | 26.6 | 81.7 | 250.7 | 367.0 |
| triangular | ML | 27.2 | 33.5 | 81.5 | 198.1 | 244.5 |
| triangular | MO | 22.6 | 28.9 | 81.7 | 231.3 | 296.0 |
| triangular | GR | 20.2 | 26.4 | 81.7 | 253.2 | 330.9 |
| burr | ML | 27.5 | 32.7 | 72.0 | 249.1 | 400.2 |

Table 16. Estimated quantiles of the fitted SSDs for Malathion Web ICE predicted LC50s based on fathead minnow.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| dist | method | HC05 | HC10 | HC50 | HC90 | HC95 |
| gumbel | ML | 1681.1 | 2030.7 | 4807.7 | 18589.5 | 31166.6 |
| gumbel | MO | 1993.7 | 2331.3 | 4758.9 | 14581.6 | 22367.7 |
| gumbel | GR | 1699.5 | 2052.0 | 4848.4 | 18687.9 | 31293.9 |
| normal | ML | 1601.8 | 2094.5 | 5393.8 | 13890.5 | 18162.6 |
| normal | MO | 1539.3 | 2030.5 | 5393.8 | 14328.1 | 18900.4 |
| normal | GR | 1285.1 | 1764.1 | 5393.8 | 16491.9 | 22639.6 |
| logistic | ML | 1495.1 | 2088.3 | 5579.1 | 14904.9 | 20819.0 |
| logistic | MO | 1564.7 | 2142.1 | 5393.8 | 13581.9 | 18593.1 |
| logistic | GR | 1203.0 | 1760.4 | 5393.8 | 16526.2 | 24184.9 |
| triangular | ML | 1694.9 | 2097.0 | 5149.4 | 12645.1 | 15644.9 |
| triangular | MO | 1504.4 | 1921.3 | 5393.8 | 15142.2 | 19338.2 |
| triangular | GR | 1338.8 | 1748.4 | 5393.8 | 16639.8 | 21731.0 |
| burr | ML | 1664.1 | 2048.5 | 4919.0 | 17836.7 | 28964.3 |

Table 17. Estimated quantiles of the fitted SSDs for Malathion Web ICE predicted LC50s based on all three surrogates.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| dist | method | HC05 | HC10 | HC50 | HC90 | HC95 |
| gumbel | ML | 23.9 | 32.8 | 137.9 | 1314.1 | 3110.1 |
| gumbel | MO | 13.8 | 21.2 | 147.7 | 3119.4 | 10004.4 |
| gumbel | GR | 10.5 | 17.0 | 151.9 | 4737.5 | 17634.1 |
| normal | ML | 7.2 | 15.1 | 207.8 | 2851.9 | 5992.3 |
| normal | MO | 6.8 | 14.5 | 207.8 | 2974.0 | 6323.4 |
| normal | GR | 5.1 | 11.6 | 207.8 | 3737.2 | 8477.8 |
| logistic | ML | 5.6 | 12.5 | 133.4 | 1419.8 | 3173.3 |
| logistic | MO | 7.1 | 16.8 | 207.8 | 2570.8 | 6047.3 |
| logistic | GR | 4.6 | 12.0 | 207.8 | 3586.8 | 9449.2 |
| triangular | ML | 6.8 | 13.0 | 203.3 | 3170.4 | 6078.3 |
| triangular | MO | 6.4 | 12.5 | 207.8 | 3457.1 | 6730.4 |
| triangular | GR | 5.4 | 10.9 | 207.8 | 3968.5 | 7982.8 |
| burr | ML | 23.9 | 32.8 | 137.9 | 1314.0 | 3109.3 |

Table 18. Estimated quantiles of the fitted SSDs for Malathion empirical LC50s for freshwater fish

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| dist | method | HC05 | HC10 | HC50 | HC90 | HC95 |
| gumbel | ML | 56.9 | 93.9 | 920.0 | 33028.5 | 129749.7 |
| gumbel | MO | 77.7 | 121.2 | 921.4 | 22212.3 | 74940.6 |
| gumbel | GR | 61.2 | 100.1 | 942.8 | 31844.0 | 122214.1 |
| normal | ML | 38.9 | 84.6 | 1315.3 | 20441.3 | 44491.3 |
| normal | MO | 37.3 | 81.9 | 1315.3 | 21132.5 | 46431.4 |
| normal | GR | 29.0 | 67.4 | 1315.3 | 25657.6 | 59561.3 |
| logistic | ML | 30.9 | 80.5 | 1343.3 | 22401.6 | 58329.7 |
| logistic | MO | 39.0 | 95.3 | 1315.3 | 18152.4 | 44318.1 |
| logistic | GR | 26.5 | 71.5 | 1315.3 | 24212.5 | 65197.8 |
| triangular | ML | 48.9 | 102.0 | 2261.5 | 50146.8 | 104507.9 |
| triangular | MO | 34.9 | 70.0 | 1315.3 | 24726.7 | 49554.0 |
| triangular | GR | 30.4 | 62.5 | 1315.3 | 27668.9 | 56947.4 |
| burr | ML | 56.9 | 93.9 | 920.4 | 33007.9 | 129594.1 |

**VI. Calculation of thresholds**

Thresholds were calculated using a probit curve with the HC05 as the mean and three different slopes (2, 4.5, and 9). Calculated thresholds are provided in Tables 19 - 23.

Table 19. Thresholds (μg/L) for determination of action area based on Web ICE predicted values of Malathion LC50s from rainbow trout

|  |  |  |  |
| --- | --- | --- | --- |
| distrib. | method | Mortality Threshold (10-6) | Indirect Effects Threshold (10-1) |
| slope = 4.5 | slope = 2 | slope = 9 | slope = 4.5 | slope = 2 | slope = 9 |
| gumbel | ML | 3.9 | 0.2 | 13.3 | 23.3 | 10.3 | 32.4 |
| gumbel | MO | 3.9 | 0.2 | 13.2 | 23.1 | 10.2 | 32.1 |
| gumbel | GR | 3.6 | 0.2 | 12.1 | 21.1 | 9.3 | 29.3 |
| normal | ML | 3.2 | 0.2 | 10.9 | 19.1 | 8.4 | 26.4 |
| normal | MO | 3.2 | 0.2 | 10.7 | 18.7 | 8.2 | 26.0 |
| normal | GR | 2.9 | 0.1 | 9.7 | 17.0 | 7.5 | 23.5 |
| logistic | ML | 3.0 | 0.1 | 10.1 | 17.8 | 7.8 | 24.6 |
| logistic | MO | 3.2 | 0.2 | 10.8 | 19.0 | 8.4 | 26.3 |
| logistic | GR | 2.8 | 0.1 | 9.3 | 16.4 | 7.2 | 22.7 |
| triangular | ML | 3.4 | 0.2 | 11.5 | 20.1 | 8.9 | 27.9 |
| triangular | MO | 3.1 | 0.1 | 10.5 | 18.4 | 8.1 | 25.5 |
| triangular | GR | 2.9 | 0.1 | 9.9 | 17.3 | 7.6 | 24.0 |
| burr | ML | 3.9 | 0.2 | 13.3 | 23.3 | 10.3 | 32.4 |

Table 20. Thresholds (μg/L) for determination of action area based on Web ICE predicted values of Malathion LC50s from bluegill

|  |  |  |  |
| --- | --- | --- | --- |
| distrib. | method | Mortality Threshold (10-6) | Indirect Effects Threshold (10-1) |
| slope = 4.5 | slope = 2 | slope = 9 | slope = 4.5 | slope = 2 | slope = 9 |
| gumbel | ML | 2.4 | 0.1 | 8.1 | 14.3 | 6.3 | 19.8 |
| gumbel | MO | 2.6 | 0.1 | 8.9 | 15.5 | 6.8 | 21.6 |
| gumbel | GR | 2.3 | 0.1 | 7.6 | 13.3 | 5.9 | 18.5 |
| normal | ML | 2.1 | 0.1 | 7.1 | 12.4 | 5.5 | 17.3 |
| normal | MO | 2.0 | 0.1 | 6.8 | 12.0 | 5.3 | 16.6 |
| normal | GR | 1.7 | 0.1 | 5.7 | 10.1 | 4.4 | 14.0 |
| logistic | ML | 1.9 | 0.1 | 6.3 | 11.0 | 4.9 | 15.3 |
| logistic | MO | 2.1 | 0.1 | 7.0 | 12.2 | 5.4 | 16.9 |
| logistic | GR | 1.6 | 0.1 | 5.4 | 9.4 | 4.2 | 13.1 |
| triangular | ML | 2.4 | 0.1 | 8.1 | 14.1 | 6.2 | 19.6 |
| triangular | MO | 2.0 | 0.1 | 6.7 | 11.7 | 5.2 | 16.2 |
| triangular | GR | 1.8 | 0.1 | 6.0 | 10.5 | 4.6 | 14.5 |
| burr | ML | 2.4 | 0.1 | 8.1 | 14.2 | 6.3 | 19.8 |

Table 21. Thresholds (μg/L) for determination of action area based on Web ICE predicted values of Malathion LC50s from fathead minnow

|  |  |  |  |
| --- | --- | --- | --- |
| distrib. | method | Mortality Threshold (10-6) | Indirect Effects Threshold (10-1) |
| slope = 4.5 | slope = 2 | slope = 9 | slope = 4.5 | slope = 2 | slope = 9 |
| gumbel | ML | 147.7 | 7.1 | 498.2 | 872.6 | 384.4 | 1211.2 |
| gumbel | MO | 175.1 | 8.4 | 590.9 | 1034.8 | 455.9 | 1436.4 |
| gumbel | GR | 149.3 | 7.1 | 503.7 | 882.1 | 388.6 | 1224.4 |
| normal | ML | 140.7 | 6.7 | 474.7 | 831.4 | 366.3 | 1154.0 |
| normal | MO | 135.2 | 6.5 | 456.2 | 799.0 | 352.0 | 1109.0 |
| normal | GR | 112.9 | 5.4 | 380.9 | 667.0 | 293.9 | 925.8 |
| logistic | ML | 131.3 | 6.3 | 443.1 | 776.0 | 341.9 | 1077.1 |
| logistic | MO | 137.4 | 6.6 | 463.7 | 812.2 | 357.8 | 1127.3 |
| logistic | GR | 105.7 | 5.1 | 356.5 | 624.4 | 275.1 | 866.7 |
| triangular | ML | 148.9 | 7.1 | 502.3 | 879.8 | 387.6 | 1221.1 |
| triangular | MO | 132.1 | 6.3 | 445.9 | 780.9 | 344.0 | 1083.9 |
| triangular | GR | 117.6 | 5.6 | 396.8 | 694.9 | 306.2 | 964.5 |
| burr | ML | 146.2 | 7.0 | 493.2 | 863.8 | 380.5 | 1198.9 |

Table 22. Thresholds (μg/L) for determination of action area based on Web ICE predicted values of Malathion LC50s from all three surrogates

|  |  |  |  |
| --- | --- | --- | --- |
| distrib. | method | Mortality Threshold (10-6) | Indirect Effects Threshold (10-1) |
| slope = 4.5 | slope = 2 | slope = 9 | slope = 4.5 | slope = 2 | slope = 9 |
| gumbel | ML | 2.1 | 0.1 | 7.1 | 12.4 | 5.5 | 17.2 |
| gumbel | MO | 1.2 | 0.1 | 4.1 | 7.2 | 3.2 | 10.0 |
| gumbel | GR | 0.9 | 0.0 | 3.1 | 5.4 | 2.4 | 7.6 |
| normal | ML | 0.6 | 0.0 | 2.1 | 3.7 | 1.6 | 5.2 |
| normal | MO | 0.6 | 0.0 | 2.0 | 3.5 | 1.6 | 4.9 |
| normal | GR | 0.4 | 0.0 | 1.5 | 2.6 | 1.2 | 3.7 |
| logistic | ML | 0.5 | 0.0 | 1.7 | 2.9 | 1.3 | 4.0 |
| logistic | MO | 0.6 | 0.0 | 2.1 | 3.7 | 1.6 | 5.1 |
| logistic | GR | 0.4 | 0.0 | 1.4 | 2.4 | 1.0 | 3.3 |
| triangular | ML | 0.6 | 0.0 | 2.0 | 3.5 | 1.6 | 4.9 |
| triangular | MO | 0.6 | 0.0 | 1.9 | 3.3 | 1.5 | 4.6 |
| triangular | GR | 0.5 | 0.0 | 1.6 | 2.8 | 1.2 | 3.9 |
| burr | ML | 2.1 | 0.1 | 7.1 | 12.4 | 5.5 | 17.2 |

Table 23. Thresholds (μg/L) for determination of action area using empirical values for Malathion LC50s for freshwater fish

|  |  |  |  |
| --- | --- | --- | --- |
| distrib. | method | Mortality Threshold (10-6) | Indirect Effects Threshold (10-1) |
| slope = 4.5 | slope = 2 | slope = 9 | slope = 4.5 | slope = 2 | slope = 9 |
| gumbel | ML | 5.0 | 0.2 | 16.9 | 29.6 | 13.0 | 41.0 |
| gumbel | MO | 6.8 | 0.3 | 23.0 | 40.3 | 17.8 | 56.0 |
| gumbel | GR | 5.4 | 0.3 | 18.1 | 31.8 | 14.0 | 44.1 |
| normal | ML | 3.4 | 0.2 | 11.5 | 20.2 | 8.9 | 28.0 |
| normal | MO | 3.3 | 0.2 | 11.0 | 19.3 | 8.5 | 26.8 |
| normal | GR | 2.6 | 0.1 | 8.6 | 15.1 | 6.6 | 20.9 |
| logistic | ML | 2.7 | 0.1 | 9.2 | 16.1 | 7.1 | 22.3 |
| logistic | MO | 3.4 | 0.2 | 11.6 | 20.3 | 8.9 | 28.1 |
| logistic | GR | 2.3 | 0.1 | 7.9 | 13.8 | 6.1 | 19.1 |
| triangular | ML | 4.3 | 0.2 | 14.5 | 25.4 | 11.2 | 35.3 |
| triangular | MO | 3.1 | 0.1 | 10.3 | 18.1 | 8.0 | 25.2 |
| triangular | GR | 2.7 | 0.1 | 9.0 | 15.8 | 6.9 | 21.9 |
| burr | ML | 5.0 | 0.2 | 16.9 | 29.5 | 13.0 | 41.0 |

**VII. Comparison of Predicted and Tested Toxicity**

To further compare SSDs fit to predicted values to SSDs generated from test results I cross-tabulated the taxa from each set of predicted values with corresponding taxa for which empirical test results were available (Tables 24 – 28). These tables also report the discrepancy as absolute value of the difference between predicted and tested toxicity value, scaled to the empirical toxicity value (e.g., the discrepancy for *Lepomis macrochirus* when *Oncorhynchus mykiss* is used as a surrogate is |108.2 – 69.3|/69.3 = 0.56) and the toxicological distance as the absolute value of the difference between the tested toxicity value of the surrogate test species and that of every other species. For example, the distance between *Oncorhynchus mykiss* and *Lepomis macrochirus*, when *Oncorhynchus mykiss* is used as the surrogate is |73.5− 69.3|/69.3 = 0.06.

Two correlations were examined for each of these sets of crosstablulated data, between the predicted and tested toxicity values and between the discrepancy statistic and the distance statistic. The latter test was done to examine whether predicted values get worse as the distance (measured as relative sensitivity to the chemical) increases between taxa.

Results of these correlations are presented in Table 28. In all cases, the predicted and tested values were significantly correlated. However, there was also a significant correlation between the discrepancy and distance statistics, suggesting that an estimated toxicity value would be less accurate, as the difference in sensitivity between surrogate and predicted species grows.

Table 24. Crosstabulated toxicity values for Web ICE predictions based on rainbow trout regressions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Taxon | Web ICE | Geomean | Discrepancy | Distance |
| Micropterus salmoides | 57.50 | 266.93 | 0.78 | 0.72 |
| Salmo salar | 66.24 | 313.60 | 0.79 | 0.77 |
| Perca flavescens | 68.09 | 263.00 | 0.74 | 0.72 |
| Sander vitreus | 70.10 | 64.00 | 0.10 | 0.15 |
| Salmo trutta | 71.43 | 101.00 | 0.29 | 0.27 |
| Oncorhynchus mykiss | 73.52 | 73.52 | 0.00 | 0.00 |
| Salvelinus namaycush | 84.62 | 103.88 | 0.19 | 0.29 |
| Oncorhynchus clarkii | 85.07 | 235.02 | 0.64 | 0.69 |
| Oncorhynchus kisutch | 99.05 | 173.46 | 0.43 | 0.58 |
| Lepomis macrochirus | 108.24 | 69.28 | 0.56 | 0.06 |
| Lepomis cyanellus | 138.81 | 163.16 | 0.15 | 0.55 |
| Ictalurus punctatus | 183.14 | 8267.49 | 0.98 | 0.99 |
| Pimephales promelas | 212.57 | 11029.17 | 0.98 | 0.99 |
| Cyprinus carpio | 229.07 | 4941.00 | 0.95 | 0.99 |
| Carassius auratus | 334.24 | 5161.09 | 0.94 | 0.99 |
| Poecilia reticulata | 378.95 | 3069.00 | 0.88 | 0.98 |
| Ameiurus melas | 392.63 | 12285.36 | 0.97 | 0.99 |

Table 25. Crosstabulated toxicity values for Web ICE predictions based on bluegill regressions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Taxon | Web ICE | Geomean | Discrepancy | Distance |
| Salmo salar | 26.3 | 313.6 | 0.92 | 0.78 |
| Salmo trutta | 36.7 | 101.0 | 0.64 | 0.31 |
| Perca flavescens | 53.6 | 263.0 | 0.80 | 0.74 |
| Micropterus salmoides | 56.8 | 266.9 | 0.79 | 0.74 |
| Oncorhynchus kisutch | 58.5 | 173.5 | 0.66 | 0.60 |
| Oncorhynchus mykiss | 63.7 | 73.5 | 0.13 | 0.06 |
| Lepomis macrochirus | 69.3 | 69.3 | 0.00 | 0.00 |
| Salvelinus namaycush | 86.8 | 103.9 | 0.16 | 0.33 |
| Oncorhynchus clarkii | 101.1 | 235.0 | 0.57 | 0.71 |
| Lepomis cyanellus | 130.7 | 163.2 | 0.20 | 0.58 |
| Pimephales promelas | 167.4 | 11029.2 | 0.98 | 0.99 |
| Cyprinus carpio | 179.4 | 4941.0 | 0.96 | 0.99 |
| Ictalurus punctatus | 219.1 | 8267.5 | 0.97 | 0.99 |
| Poecilia reticulata | 234.0 | 3069.0 | 0.92 | 0.98 |
| Carassius auratus | 274.5 | 5161.1 | 0.95 | 0.99 |

Table 26. Crosstabulated toxicity values for Web ICE predictions based on fathead minnow regressions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Taxon | Web ICE | Geomean | Discrepancy | Distance |
| Salvelinus namaycush | 1374.7 | 103.9 | 12.2 | 105.2 |
| Oncorhynchus kisutch | 1740.1 | 173.5 | 9.0 | 62.6 |
| Perca flavescens | 2351.2 | 263.0 | 7.9 | 40.9 |
| Micropterus salmoides | 2728.5 | 266.9 | 9.2 | 40.3 |
| Oncorhynchus clarkii | 2730.3 | 235.0 | 10.6 | 45.9 |
| Oncorhynchus mykiss | 4615.0 | 73.5 | 61.8 | 149.0 |
| Lepomis macrochirus | 6234.6 | 69.3 | 89.0 | 158.2 |
| Cyprinus carpio | 6520.4 | 4941.0 | 0.3 | 1.2 |
| Poecilia reticulata | 10520.7 | 3069.0 | 2.4 | 2.6 |
| Pimephales promelas | 11029.2 | 11029.2 | 0.0 | 0.0 |
| Carassius auratus | 11101.7 | 5161.1 | 1.2 | 1.1 |
| Ameiurus melas | 11480.5 | 12285.4 | 0.1 | 0.1 |
| Oryzias latipes | 12110.6 | 9700.0 | 0.2 | 0.1 |
| Ictalurus punctatus | 15639.9 | 8267.5 | 0.9 | 0.3 |

Table 27. Crosstabulated toxicity values for Web ICE predictions based on all three surrogates.

|  |  |  |
| --- | --- | --- |
| Taxon | Web ICE | Geomean |
| Micropterus salmoides | 56.8 | 266.9 |
| Salmo salar | 66.2 | 313.6 |
| Perca flavescens | 68.1 | 263.0 |
| Lepomis macrochirus | 69.3 | 69.3 |
| Sander vitreus | 70.1 | 64.0 |
| Salmo trutta | 71.4 | 101.0 |
| Oncorhynchus mykiss | 73.5 | 73.5 |
| Salvelinus namaycush | 84.6 | 103.9 |
| Oncorhynchus clarkii | 85.1 | 235.0 |
| Oncorhynchus kisutch | 99.1 | 173.5 |
| Lepomis cyanellus | 130.7 | 163.2 |
| Ictalurus punctatus | 183.1 | 8267.5 |
| Cyprinus carpio | 6520.4 | 4941.0 |
| Poecilia reticulata | 10520.6 | 3069.0 |
| Pimephales promelas | 11029.2 | 11029.2 |
| Carassius auratus | 11101.7 | 5161.1 |
| Ameiurus melas | 11480.4 | 12285.4 |
| Oryzias latipes | 12110.6 | 9700.0 |

Table 28. Correlations between cross-tabulated toxicity values

|  |  |  |
| --- | --- | --- |
| Surrogate Species | correlation between predicted and tested | Correlation between discrepancy and distance |
| rainbow trout | ρ = 0.74, p = 7.5\*10-4 | ρ = 0.88, p = 2.6\*10-6 |
| bluegill | ρ = 0.72, p = 2.7\*10-3 | ρ = 0.90, p = 4.6\*10-6 |
| fathead minnow | ρ = 0.84, p = 1.8\*10-4 | ρ = 0.89, p = 1.7\*10-5 |
| all three | ρ = 0.80, p = 1.4\*10-4 | n/a |