**APPENDIX 2-5. Methomyl Species Sensitivity Distribution Analysis for Fish**

SSDs were fit to toxicity data for freshwater and saltwater fish and amphibians exposed to methomyl. Five distributions were tested and a variety of methods were used to determine whether different subsets of data should be modeled independently. Amphibian and saltwater fish mortality data were insufficient to derive separate SSDs. Ultimately, the results from the all vertebrates SSDs were used to represent freshwater fish, saltwater fish, and amphibians.  **Table 1** provides a summary of the results.

**Table 1. Summary statistics for SSDs fit to methomyl test results.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Statistic | AllVertebrates | FWVertebrates | AllFish | FWFish |
| Best Distribution (by AICc) | gumbel | gumbel | triangular | triangular |
| Goodness of fit P-value | 1.00 | 0.999 | 1.00 | 1.00 |
| CV of the HC05 | 0.452 | 0.546 | 0.239 | 0.274 |
| HC05 | 335 | 312 | 472 | 472 |
| HC10 | 462 | 446 | 560 | 564 |
| HC50 | 2010 | 2300 | 1160 | 1200 |
| HC90 | 20200 | 30100 | 2400 | 2540 |
| HC95 | 49000 | 80400 | 2850 | 3030 |

1. Data

Data used in this analysis were from an ECOTOX query (**APPENDIX 2-2**) plus data from submitted studies are detailed in **Tables 15** and **16** (end of document). **Table 2** provides the distribution of the test results for methomyl including the number of species represented. Since only two saltwater species were tested and three amphibians were tested, insufficient data were available to derive separate SSDs for these two taxa. Two sets of distributions were derived: freshwater fish and all vertebrates (combines freshwater fish, saltwater fish and amphibians).

**Table 2. Distribution of test results available for methomyl.**

|  |  |  |
| --- | --- | --- |
| Data Subset | Test results | Species |
| All Aquatic Vertebrates | 56 | 16 |
| Freshwater Vertebrates | 47 | 14 |
| All Fish1  | 43 | 13 |
| Freshwater Fish | 34 | 11 |
| Saltwater Fish | 9 | 2 |
| Aquatic Amphibians | 13 | 3 |

**Figure 1** shows the distribution of test results among species, indicating that a few species have been repeatedly tested (three species have been tested at least 8 times each), but the majority of species have been tested fewer than six times, with 7 species having only one test result.

 

**Figure 1. Distribution of the number of test results per species in Methomyl aquatic vertebrate data.**

Five potential distributions for the methomyl data were considered, including log-normal, log-logistic, log-triangular, log-gumbel, and Burr. To fit each of the first four distributions, the toxicity values were first common log (log10) transformed. Finally, effect thresholds and five quantiles from the fitted SSDs (HC05, HC10, HC50, HC90, HC95) were calculated and reported.

1. Comparison of distributions using AICc

Akaike’s Information Criterion corrected for sample size (AICc) was used to compare the five distributions for all four datasets. For these comparisons all SSDs were fit using maximum likelihood. For all of the datasets that included amphibians, AICc suggested that the triangular distribution provided the best fit (**Tables 3** and **4**). For all fish and freshwater fish, AICc suggested that the gumbel distribution provided the best fit (**Table 5** and **6**).

**Table 3. Comparison of distributions for all aquatic vertebrate toxicity data for methomyl.**

| distribution | AICc | ∆AICc | Weight | HC05 |
| --- | --- | --- | --- | --- |
| gumbel | 323.5 | 0 | 0.80 | 335 |
| burr | 326.6 | 3.09 | 0.17 | 334 |
| logistic | 330.8 | 7.33 | 0.02 | 78 |
| normal | 332.8 | 9.28 | 0.01 | 82 |
| triangular | 333.4 | 9.91 | 0.01 | 59 |

**Table 4. Comparison of distributions for freshwater vertebrate toxicity data for methomyl.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| distribution | AICc | ∆AICc | Weight | HC05 |
| gumbel | 290.8 | 0 | 0.77 | 312 |
| burr | 294.2 | 3.32 | 0.15 | 311 |
| logistic | 296.9 | 6.03 | 0.04 | 66 |
| normal | 297.8 | 6.93 | 0.02 | 80 |
| triangular | 297.8 | 6.98 | 0.02 | 66 |

**Table 5. Comparison of distributions for pooled fish toxicity data for methomyl.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| distribution | AICc | ∆AICc | Weight | HC05 |
| triangular | 208.4 | 0 | 0.36 | 472 |
| normal | 209.1 | 0.72 | 0.25 | 459 |
| logistic | 209.4 | 1.05 | 0.21 | 446 |
| gumbel | 210.2 | 1.86 | 0.14 | 490 |
| burr | 212.8 | 4.46 | 0.04 | 467 |

**Table 6. Comparison of distributions for freshwater fish toxicity data for methomyl.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| distribution | AICc | ∆AICc | Weight | HC05 |
| triangular | 179.3 | 0 | 0.48 | 472 |
| logistic | 180.6 | 1.32 | 0.25 | 415 |
| gumbel | 181.1 | 1.74 | 0.20 | 468 |
| burr | 184.6 | 5.22 | 0.04 | 433 |
| normal | 185.2 | 5.86 | 0.03 | 223 |

1. Test for the need to model results separately by freshwater fish vs. all aquatic vertebrates

Examination of the cumulative distribution functions plotted on similar axes for all vertebrates versus freshwater-only vertebrates does not lend support to modeling the datasets separately. The 95% bootstrap confidence intervals for the separate distributions overlap and are not very different (**Figure 2**). The confidence limits on the HC05 for both separate distributions are relatively precise, with the upper confidence limit falling at the 22nd and 24th percentile, respectively **(Tables 9** and **10**). Also, in both cases the CV of the HC05 is below 1. When looking at the endpoints of interest (*i.e.,* HC05 and HC50) across the SSDs for all vertebrates and freshwater fish, the percentile differences are not great, however, it is noted that a visual inspection of the plot indicates a divergence at the upper end (70th percentile) of the SSDs. Given the overlap of the confidence intervals of the distributions around the HC05 and HC50, the all vertebrate distribution is used.



**Figure 2. SSDs for combined and freshwater-only vertebrate LC50s (gumbel), and combined and freshwater-only fish LC50s (triangular) for methomyl**. [Black lines show the distribution and upper and lower confidence interval for the combined aquatic vertebrate distribution. Red lines show the upper and lower confidence interval for freshwater-only vertebrates. Green lines show the distribution and upper and lower confidence interval for all fish combined. Blue lines show the upper and lower confidence intervals for freshwater-only fish].

1. Goodness of fit

Plots of the cumulative distribution functions for the best-fit distributions (as determined by AICc) suggest little evidence of lack-of-fit (**Figs. 3, 4, 5** and **6**). Similarly, bootstrap goodness-of-fit tests did not show evidence for lack-of-fit (P-values > 0.05, **Tables 7, 8, 9** and **10**), with the exception of the Burr distribution, which frequently showed significant lack-of-fit. In general, the coefficient of variation for the HC05 was below 1 for the competitive distributions.



**Figure 3. Log-gumbel SSD for methomyl toxicity values for all aquatic vertebrates pooled.** Black points indicate single toxicity values. Red points indicate average of multiple toxicity values for a single species. Blue line indicates full range of toxicity values for a given taxon.



**Figure 4. Log-gumbel SSD for methomyl toxicity values for freshwater aquatic vertebrates pooled.** Black points indicate single toxicity values. Red points indicate average of multiple toxicity values. Blue line indicates full range of toxicity values for a given species.



**Figure 5. Log-triangular SSD for methomyl LC50s for all fish.** Black points indicate single toxicity values. Red points indicate average of multiple toxicity values for a single species. Blue line indicates full range of toxicity values for a given species.



**Figure 6. Log-triangular SSD for methomyl LC50s for freshwater fish.** Red points indicate single toxicity values. Black points indicate average of multiple toxicity values for a single species. Blue line indicates full range of toxicity values for a given species.

**Table 7. Range of HC05 values for methomyl SSDs for all aquatic vertebrates.**

****

ML=maximum likelihood, MO= moment estimators, and GR=graphical methods

LCp and UCp=projections of the confidence limits of the HC05 (LCx and UCx) onto the cumulative distribution function of the fitted distribution.

**Table 8. Range of HC05 values for methomyl SSDs for freshwater vertebrates.**



ML=maximum likelihood, MO= moment estimators, and GR=graphical methods

LCp and UCp=projections of the confidence limits of the HC05 (LCx and UCx) onto the cumulative distribution function of the fitted distribution.

**Table 9. Range of HC05 values for methomyl SSDs for all fish pooled.**

****

ML=maximum likelihood, MO= moment estimators, and GR=graphical methods

LCp and UCp=projections of the confidence limits of the HC05 (LCx and UCx) onto the cumulative distribution function of the fitted distribution.

**Table 10. Range of HC05 values for methomyl SSDs for freshwater fish.**

****

ML=maximum likelihood, MO= moment estimators, and GR=graphical methods

LCp and UCp=projections of the confidence limits of the HC05 (LCx and UCx) onto the cumulative distribution function of the fitted distribution.

1. Calculation of other quantiles

**Tables 11, 12, 13** and **14** provide estimates of the HC05 as well as other quantiles of the fitted SSDs.

**Table 11. Estimated quantiles of the fitted SSDs for methomyl LC50s for all aquatic vertebrates.**

****

**Table 12. Estimated quantiles of the fitted SSDs for methomyl LC50s for freshwater aquatic vertebrates.**



**Table 13. Estimated quantiles of the fitted SSDs for Methomyl LC50s for all fish.**

****

**Table 14. Estimated quantiles of the fitted SSDs for methomyl LC50s for freshwater fish.**

****

**Tables 15** and **16** provide all of the available LC50 values for fish and amphibians, respectively (within the 96-hour timeframe-unless otherwise noted). These data sets are the same as reported in the effects characterization and provide additional data for the formulated products. Values that were included in the SSD (*i.e.,* TGAI) are marked with an \*.

**Table 15. Available median lethal concentration (LC50) data for fish exposed to methomyl as TGAI or formulation.**

| **Family** | **Species** | **Common Name** | **LC50 (µg/L)1** | **MRID/ ECOTOX Ref. #** | **Classification** |
| --- | --- | --- | --- | --- | --- |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 370 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 430 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 480\* | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 560 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 560 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 600\* | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 600 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 620\* | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 670 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 670 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 710 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 840\* | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 860\* | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 875\* | E5395  | NA |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 940\* | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 1050\* | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 1150\* | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 1200\* | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 1200 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 1800 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 2000\* | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Lepomis macrochirus* | Bluegill Sunfish | 2800 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Micropterus salmoides* | Largemouth Bass | 760 | MRID 40098001/E6797 | Supplemental |
| Centrarchidae | *Micropterus salmoides* | Largemouth Bass | 1250\* | MRID 40098001/E6797 | Supplemental |
| Cichlidae | *Oreochromis mossambicus* | Mozambique Tilapia | 880\* | E110907 | NA |
| Cichlidae | *Oreochromis niloticus* | Nile Tilapia | 1070\* | E16196 | NA |
| Cichlidae | *Oreochromis niloticus* | Nile Tilapia | 1630\* | E118556 | NA |
| Cyprinidae | *Cyprinus carpio* | Common Carp | 3550\* | MRID 48986701 | Supplemental/Quantitative |
| Cyprinidae | *Pimephales promelas* | Fathead Minnow | 1500\* | MRID 40098001/E6797 | Supplemental |
| Cyprinidae | *Pimephales promelas* | Fathead Minnow | 1800 | MRID 40098001/E6797 | Supplemental |
| Cyprinidae | *Pimephales promelas* | Fathead Minnow | 2800\* | MRID 40098001/E6797 | Supplemental |
| Cyprinidae | *Pseudorasbora parva* | Topmouth gudgeon | 417\* | E110202 | Quantitative – for SSD |
| Cyprinodontidae |  *Cyprinodon variegates* | Sheepshead Minnow | 1160\* | MRID 41441202 | Acceptable |
| Ictaluridae | *Ictalurus punctatus* | Channel Catfish | 320 | MRID 40098001/E6797 | Supplemental |
| Ictaluridae | *Ictalurus punctatus* | Channel Catfish | 530\* | MRID 40098001/E6797 | Supplemental |
| Ictaluridae | *Ictalurus punctatus* | Channel Catfish | 760 | MRID 40098001/E6797 | Supplemental |
| Ictaluridae | *Ictalurus punctatus* | Channel Catfish | 1800 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhynchus clarkii* | Cutthroat Trout  | 760\* | MRID 40094602 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 860\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1050\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1100\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1200\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1200 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1200 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1300 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1400\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1400 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1400 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1400 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1400 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1500\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1500 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1600\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 1700\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 2000\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 2000 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 2100 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 2300 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 3200 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 3400\* | E5395  | NA |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 4040\* | E118556 | NA |
| Salmonidae | *Oncorhyncus mykiss* | Rainbow Trout | 32,000 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salmo salar* | Atlantic Salmon | 560\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salmo salar* | Atlantic Salmon | 640\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salmo salar* | Atlantic Salmon | 700\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salmo salar* | Atlantic Salmon | 1000\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salmo salar* | Atlantic Salmon | 1050\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salmo salar* | Atlantic Salmon | 1120\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salmo salar* | Atlantic Salmon | 1150\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salmo salar* | Atlantic Salmon | 1200 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salmo salar* | Atlantic Salmon | 1220\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salmo salar* | Atlantic Salmon | 1400 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salvelinus fontinalis* | Brook Trout | 1220 | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salvelinus fontinalis* | Brook Trout | 1500\* | MRID 40098001/E6797 | Supplemental |
| Salmonidae | *Salvelinus fontinalis* | Brook Trout | 2200\* | MRID 40098001/E6797 | Supplemental |

\* Indicates study was conducted with TGAI and value used to derive SSD.

 NA = Studies are acceptable for ECOTOX but have not been formally reviewed by EFED scientists.

1If a species was not represented with a 96 hour study, and other values were available from shorter/longer duration studies (up to 10 days) then the data were included and the duration was listed next to the LC50 value as an indicator. If there were multiple other durations, the value from the study closer to a 96h duration was selected and if there were multiple values for the same duration, the range is indicated.

**Table 16. Available median lethal concentration (LC50) data for amphibians exposed to methomyl as TGAI or formulation.**

| **Family** | **Species** | **Common Name** | **LC50 (µg/L)1** | **Reference Number** | **Classification** |
| --- | --- | --- | --- | --- | --- |
| Bufonidae | *Polypedates melanostictus* | Asian Common Toad | 848,000\*15°C | E171543 | NA2 |
| Bufonidae | *Polypedates melanostictus* | Asian Common Toad | 941,000\*25°C | E171543 | Quantitative2 |
| Bufonidae | *Polypedates melanostictus* | Asian Common Toad | 378,000\*30°C | E171543 | NA2 |
| Bufonidae | *Polypedates melanostictus* | Asian Common Toad | 266,000\*35°C | E171543 | NA2 |
| Bufonidae | *Polypedates melanostictus* | Asian Common Toad | 1,110,000\*20°C | E171543 | NA2 |
| Microhylidae | *Microhyla pulchra* | Marbled Pygmy Frog | 97,700\*15°C | E171543 | Qualitative |
| Microhylidae | *Microhyla pulchra* | Marbled Pygmy Frog | 88,800\*20°C | E171543 | Qualitative |
| Microhylidae | *Microhyla pulchra* | Marbled Pygmy Frog | 46,600\*30°C | E171543 | Qualitative |
| Microhylidae | *Microhyla pulchra* | Marbled Pygmy Frog | 84,600\*25°C | E171543 | Quantitative– for Threshold Use |
| Microhylidae | *Microhyla pulchra* | Marbled Pygmy Frog | 15,400\*35°C | E171543 | Qualitative |
| Rhacophoridae | *Polypedates megacephalus* | Tree Frog | 467,000\*20°C | E171543 | NA2 |
| Rhacophoridae | *Polypedates megacephalus* | Tree Frog | 380,000\*25°C | E171543 | Quantitative2 |
| Rhacophoridae | *Polypedates megacephalus* | Tree Frog | 449,000\*30°C | E171543 | NA2 |
| Rhacophoridae | *Polypedates megacephalus* | Tree Frog | 35°C | E171543 | Invalid3 |

\* Indicates study was conducted with TGAI and value used to derive SSD.

 NA = Studies are acceptable for ECOTOX but have not been formally reviewed by EFED scientists.

1 If a species was not represented with a 96 hour study, and other values were available from shorter/longer duration studies (up to 10 days) the data were included and the duration was listed next to the LC50 value as an indicator. If there were multiple other durations, the value from the study closer to a 96 hour duration was selected and if there were multiple values for the same duration, the range is indicated. These data also have temperature notations due to the particular study design; in one case a 48-hour data point with a different temperature regime is also included despite having 96-hour data available, to display the range.

2 For the Asian Common Toad and the Tree Frog, the temperature ranges should be confirmed in order to use their endpoints quantitatively. The temperature range was only confirmed for the Marbled Pygmy Frog for the review since one of those endpoints was a candidate for threshold use.

3 96-hour LC50 for the brown treefrog from the 35ºC treatment due to high (30%) control mortality.