**APPENDIX 2-5. Imidacloprid Species Sensitivity Distribution Analysis for Aquatic Invertebrates**

# **Summary**

Species Sensitivity Distributions (SSDs) were fit to median lethal or effects (immobility) concentrations (LC50 or EC50 values, respectively) for aquatic invertebrates exposed to imidacloprid. Separate aquatic freshwater and estuarine/marine (saltwater) invertebrate SSDs were developed. Additionally, to investigate potential differences in sensitivity among insect species and other aquatic invertebrate species, a separate freshwater aquatic insect SSD was also developed. In previous biological evaluations for other insecticides, separate SSDs for mollusks were also developed; however, this was not done for imidacloprid due to a lack of data.

Six distributions (normal, logistic, triangular, gumbel, weibull and burr) were fit to the available toxicity data for freshwater invertebrates and insects, and saltwater invertebrates. For freshwater invertebrates and freshwater insects, the triangular distribution provided the best fit for the datasets (**Figure 1**). For saltwater invertebrates the gumbel distribution provided the best fit for the dataset **Figure 2**). The distribution selection decision was based on the AICc weight, confidence limits for the different distributions (especially around the HC05 and HC50) and by visually examining the distributions and their consistency with the toxicity data. The comparison of SSDs for freshwater invertebrates and insects did not indicate a significant difference in distributions at the HC05. Therefore, the triangular distribution for all freshwater invertebrates was determined to be the best fit for the imidacloprid toxicity dataset (**Figure 1**). Summary statistics from the fitted SSDs are provided below in **Table 1**. The fifth and fiftieth percentiles of the SSD (abbreviated HC05 and HC50, respectively, where “HC” stands for “hazard concentration”) are used to calculate mortality endpoints representing effects to listed species of aquatic invertebrates associated with their prey, pollination, habitat and dispersal (PPHD).

**Table 1. Summary of imidacloprid mortality endpoints for aquatic invertebrates (values in µg a.i./L).**

|  |  |  |
| --- | --- | --- |
| Statistic | Freshwater Invertebrates | Saltwater Invertebrates |
| HC05 (95% CI) | 1.43 (0.71-5.54) | 13.15 (2.11-204.52) |
| HC50 (95% CI) | 154 (71.8-331.5) | 636.25 (96.88-8217) |
| Median Slope | 1.7 | 3.63 |

CI = confidence interval



**Figure 1. Normal SSD for imidacloprid toxicity values for freshwater aquatic invertebrates.**



**Figure 2. Gumbel SSD for imidacloprid toxicity values for saltwater aquatic invertebrates.**

# **Toxicity Data**

Because an SSD depicts relative sensitivities of different species exposed to the same stressor, it is necessary to standardize the data as much as possible to eliminate variables that would confound the relative sensitivities of species. Such variables can include study exposure duration, age class of organisms tested, and other study design factors. The EC/LC50 values that were included in the analysis were all definitive mortality or immobility endpoints from either 48 or 96-hour tests[[1]](#footnote-2), with a minimum of four concentrations of technical grade active ingredient, plus appropriate controls, tested within each study. Additionally, if a definitive immobility and mortality endpoint was available from the same test, the mortality endpoint was used (because immobility is intended as a surrogate for mortality). In some cases, only the genus was available, so the species is unknown. Endpoints without definitive endpoints were not used to derive SSDs.

Data used to derive SSDs are from literature that passed the ECOTOX quality screen (catalogued in **APPENDIX 2-2**) and data from unpublished, registrant-submitted studies. There was a total of 41 freshwater invertebrates tested, 28 of which were insects, and 8 saltwater aquatic invertebrate species **(Table 2**). Note that for some of the species in Raby et al. 2018 (178290), wild caught species were only identified down to the genus level; however, they are assumed to represent the same species. For all species, there are one to seven different toxicity endpoints (LC50 or EC50 values) available. In cases where multiple endpoints were available for the same test species, the full range of applicable data is used in SSD creation. Using the available data, the slope was calculated for all aquatic invertebrates resulting in a median slope of 1.7. **Table 3** and **Table 4** show the data used in SSD creation for freshwater and saltwater invertebrates, respectively.

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

|  |  |  |
| --- | --- | --- |
| Media | Test results | Species |
| Freshwater invertebrates | 65 | 41 |
| Freshwater insects | 38 | 28 |
| Freshwater non-insects | 27 | 13 |
| Saltwater invertebrates | 11 | 8 |

**Table 3. Test results used to derive SSDs for imidacloprid for freshwater aquatic invertebrates.**

| **Genus or Species** | **Acute EC/LC50 value (µg/L)** | **Slope** | **Reference (ECOTOX #)** |
| --- | --- | --- | --- |
| *Epeorus longimanus\** | 0.65 | NA | 102580 |
| *Cloeon dipterum\** | 0.77 | 1.18 | 166772 |
| *Caenis horaria\** | 1.40 | 1.59 | 166772 |
| *Chironomus dilutus\** | 2.65 | NA | 160293 |
| *Chironomus tentans\** | 2.65 | NA | 167874 |
| *Chironomus dilutus\** | 3.56 | NA | 184107 |
| *Chironomus dilutus\** | 4.63 | NA | 183458 |
| *Cheumatopsyche brevilineata\** | 4.851 | NA | 152279 |
| *Cheumatopsyche brevilineata\** | 5.241 | NA | 152279 |
| *Neocloeon triangulifer\** | 5.62 | 3.98 | 178290 |
| *Simulium vittatum\** | 6.75 | NA | 81392 |
| *Simulium vittatum\** | 8.25 | NA | 81392 |
| *Simulium vittatum\** | 9.54 | NA | 81392 |
| *Chironomus dilutus\** | 14.2 | 1.54 | 178290 |
| *Gammarus roeseli* | 14.21 | 1.87 | 160124 |
| *Notonecta sp. \** | 171 | 1.53 | 166772 |
| *Micrasema sp. \** | 17.5 | 3.84 | 178290 |
| *Isonychia bicolor\** | 18.8 | NA | 183979 |
| *Chironomus riparius\** | 19.9 | NA | 165043 |
| *Coloburiscus humeralis\** | 31.7 | NA | 184001 |
| *Deleatidium sp. \** | 40.6 | NA | 184001 |
| *Plea minutissima\** | 42.9 | 1.84 | 166772 |
| *Lumbriculus variegatus* | 43.3 | NA | 178290 |
| *Aedes sp. \** | 44 | 4.11 | 178290 |
| *Sialis lutaria\** | 50.61 | NA | 166772 |
| *Hyalella azteca* | 56 | NA | MRID42256303 |
| *Chironomus dilutus\** | 68.9 | 1.69 | MRID42256304 |
| *Trichocorixa sp. \** | 69.3 | NA | 178290 |
| *Gammarus fossarum* | 70 | NA | 152830 |
| *Ephemerella sp. \** | 71.2 | 2.03 | 178290 |
| *Gammarus pulex* | 109.9 | NA | 153561 |
| *Asellus aquaticus* | 115 | NA | 166772 |
| *Gyrinus sp. \** | 154 | 5.52 | 178290 |
| *Chaoborus obscuripes\** | 236 | 1.4 | 166772 |
| *Culex pipiens\** | 323 | 6.7 | 184514 |
| *Aedes aegypti\** | 360 | NA | 168249 |
| *Hyalella azteca* | 362 | NA | 178290 |
| *Ceriodaphnia dubia* | 571.61 | NA | 157952 |
| *Stenelmis sp. \** | 620 | NA | 178290 |
| *Isonychia bicolor\** | 702 | 1.67 | 178290 |
| *Culex pipiens\** | 705 | 1.5 | 184514 |
| *Gammarus fossarum* | 800 | NA | 150031 |
| *Hexagenia sp. \** | 900 | NA | 183503 |
| *Daphnia pulex* | 1090 | NA | 183047 |
| *Cloeon sp. \** | 1310 | 1.28 | 178290 |
| *Asellus aquaticus* | 1517 | NA | 183972 |
| *Tubifex tubifex* | 1663 | NA | 184000 |
| *Maccaffertium sp. \** | 1840 | 2.99 | 178290 |
| *Gammarus pulex* | 3857 | NA | 153560 |
| *Ceriodaphnia reticulata* | 5552.91 | NA | 157952 |
| *Coenagrion sp. \** | 6250 | NA | 178290 |
| *Asellus aquaticus* | 8500 | NA | 150031 |
| *Asellus aquaticus* | 8500 | NA | 152830 |
| *Hexagenia sp. \** | 12000 | 0.979 | 178290 |
| *Daphnia magna* | 16200 | NA | 184090 |
| *Caecidotea sp.* | 200001 | NA | 178290 |
| *Daphnia pulex* | 368721 | NA | 157952 |
| *Daphnia magna* | 432651 | NA | 157952 |
| *Moina macrocopa* | 452711 | NA | 157952 |
| *Ceriodaphnia dubia* | 50000 | NA | 178290 |
| *Daphnia magna* | 566001 | 1.48 | 150163 |
| *Daphnia magna* | 852001 | 2.61 | MRID42055317 |
| *Daphnia magna* | 906801 | NA | 162193 |
| *Daphnia magna* | 938801 | NA | 171489 |
| *Daphnia magna* | 97000 | NA | 159937 |

NA = Not available

\*Species is in the Insecta class. A separate SSD for insects was also developed.

1EC50 value

**Table 4. Test results u sed to derive SSDs for imidacloprid for saltwater aquatic invertebrates.**

| **Genus or Species** | **Acute EC/LC50 value (µg/L)** | **Slope** | **Reference (ECOTOX #)** |
| --- | --- | --- | --- |
| *Aedes taeniorhynchus* | 13 | 3.63 | 19639 |
| *Aedes taeniorhynchus* | 21 | NA | 19639 |
| *Americamysis bahia* | 33 | 4.2 | MRID42055319 |
| *Americamysis bahia* | 36 | NA | MRID42528301 |
| *Artemia sp.* | 361230 | 3.47 | 19639 |
| *Crassostrea virginica* | 145000 | NA | MRID42256305 |
| *Lepeophtheirus salmonis* | 97.6 | NA | 175427 |
| *Lepeophtheirus salmonis* | 8400 | NA | 175427 |
| *Mytilus galloprovincialis* | 1800 | NA | 166568 |
| *Palaemonetes pugio* | 308.8 | NA | 102582 |
| *Palaemonetes pugio* | 563.5 | NA | 102582 |
| *Penaeus monodon* | 175 | NA | 184559 |

# **Determining distributions with best fit**

Six potential distributions for the imidacloprid data were considered (i.e., normal, logistic, triangular, gumbel, weibull and burr). To fit each of the six distributions, the toxicity values were common log (log10) transformed. The SSD toolbox includes four different fitting methods (*i.e.*, maximum likelihood, moment estimators, linearization and metropolis-hastings). All six distributions were fit using the maximum likelihood (ML) method. To test goodness-of-fit, all six distributions were fit to the imidacloprid data and bootstrap goodness-of-fit tests were run with 10,000 replicates. The results of these fitting exercises are presented in **Table 5** For the freshwater invertebrate and insect SSD as well as saltwater invertebrate SSD, for all distributions the p-value indicates acceptable distributions.

**Table 5. P-values calculated for SSDs for freshwater and saltwater aquatic invertebrate toxicity data for imidacloprid.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Distribution | Freshwater Inverts | Insects | Non-insects | Saltwater Inverts |
| Normal | 0.56 | 0.93 | 0.68 | 0.22 |
| Logistic | 0.34 | 0.88 | 0.60 | 0.34 |
| Triangular | 0.79 | 0.98 | 0.81 | 0.20 |
| Gumbel | 0.76 | 0.74 | 0.43 | 0.56 |
| Weibull | 0.36 | 0.25 | >0.01 | 0.56 |
| Burr | 0.62 | 0.91 | 0.57 | 0.51 |

# **Akaike’s Information Criteria (AICc) weights**

Akaike’s Information Criterion corrected for sample size (AICc) was used to compare the six distributions for the freshwater and saltwater invertebrate data at the HC052. For all freshwater invertebrates, the majority of the weight is attributed to the triangular distribution method (**Table 6**). A similar pattern was seen for freshwater insects the top distribution the triangular method carries the majority of the weight (**Table 7**). Based on the AIC weights, the fit of the triangular distribution is further considered below for all freshwater invertebrate and insect data.

**Table 6. Akaike’s Information Criteria (AICc) for distributions for freshwater invertebrate toxicity data.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Distribution | AICc | Delta AICc | Wt | HC05 | SE HC05 |
| triangular | 616.4142 | 0 | 0.6472 | 1.4295 | 0.5123 |
| normal | 618.8981 | 2.4840 | 0.1869 | 1.2154 | 0.8513 |
| gumbel | 620.9325 | 4.5183 | 0.0676 | 1.9336 | 0.8931 |
| logistic | 621.2577 | 4.8435 | 0.0575 | 0.8614 | 0.6819 |
| burr | 622.4491 | 6.0350 | 0.0317 | 1.7034 | 1.1566 |
| weibull | 624.9395 | 8.5253 | 0.0091 | 0.1419 | 0.1730 |

**Table 7. Akaike’s Information Criteria (AICc) for distributions for freshwater insect toxicity data.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Distribution | AICc | Delta AICc | Wt | HC05 | SE HC05 |
| triangular | 349.1710 | 0 | 0.6058 | 1.1017 | 0.3821 |
| normal | 351.6289 | 2.4578 | 0.1773 | 0.9470 | 0.6654 |
| logistic | 352.9736 | 3.8026 | 0.0905 | 0.7568 | 0.5949 |
| gumbel | 353.4465 | 4.2755 | 0.0714 | 1.3425 | 0.6224 |
| burr | 355.0928 | 5.9218 | 0.0314 | 1.1362 | 0.8670 |
| weibull | 355.6519 | 6.4809 | 0.0237 | 0.1632 | 0.1976 |

**Table 8. Akaike’s Information Criteria (AICc) for distributions for freshwater non-insect toxicity data.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Distribution | AICc | Delta AICc | Wt | HC05 | SE HC05 |
| triangular | 256.5406 | 0 | 0.4587 | 32.4291 | 23.9563 |
| normal | 257.6587 | 1.1180 | 0.2623 | 26.0949 | 27.7743 |
| logistic | 258.5306 | 1.9900 | 0.1696 | 22.6627 | 28.5961 |
| gumbel | 259.9900 | 3.4493 | 0.0818 | 29.2390 | 22.1367 |
| burr | 262.1580 | 5.6174 | 0.0277 | 22.8141 | 30.9941 |

For saltwater invertebrates three distributions carry the majority of the weight (**Table 8**). Based on the AIC weights, the fit of the gumbel, triangular, and normal distributions are further considered below for saltwater invertebrate data.

**Table 9. Akaike’s Information Criteria (AICc) for distributions for saltwater invertebrate toxicity data.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Distribution | AICc | Delta AICc | Wt | HC05 | SE HC05 |
| gumbel | 155.9726 | 0 | 0.3851 | 10.9818 | 11.6170 |
| triangular | 156.9352 | 0.9626 | 0.2380 | 6.0380 | 12.6883 |
| normal | 157.6739 | 1.7013 | 0.1645 | 2.8801 | 5.4560 |
| logistic | 158.1471 | 2.1745 | 0.1298 | 1.4089 | 2.8796 |
| weibull | 159.7168 | 3.7442 | 0.0592 | 0.1253 | 0.4415 |
| burr | 161.5761 | 5.6036 | 0.0234 | 10.9837 | 11.5568 |
|  |  |  |  |  |  |

# **Distributions**

The cumulative distribution functions for freshwater and saltwater invertebrate SSDs for the respective distributions are discussed in this section.

## Freshwater invertebrates

**Figure 3** depicts the triangular distribution fit to the EC/LC50 values for freshwater invertebrate species (and genera). **Table 10** includes the HC05 and HC50 values for this distribution, along with the associated 95% confidence intervals. As depicted in the figure, the lowest available toxicity value (i.e., 0.65 ug a.i./L for *Epeorus longimanus;* ECOTOX # 102580) is lower than the estimated HC05 value (**Table 10**).

**Table 10. HC05 and HC50 values (in µg a.i./L) for triangular distribution based on freshwater invertebrate EC/LC50 values.**

|  |  |  |
| --- | --- | --- |
| Distribution | HC05 (95% CI) | HC50 (95% CI) |
| Triangular | 1.43 (0.71-5.54) | 154 (71.8-331.5) |



**Figure 3. Triangular SSD for imidacloprid toxicity values for freshwater aquatic invertebrates.**

## Freshwater insects

**Figure 4** depicts the triangular distribution fit to the EC/LC50 values for freshwater insect species. **Table 11** includes the HC05 and HC50 values for this distribution, along with the associated 95% confidence intervals. As depicted in **Figure 4** the lowest available toxicity value (i.e., 0.65 ug a.i./L for *Epeorus longimanus;* ECOTOX # 102580) is less than all estimated HC05 values.The lowest available toxicity value is within the 95% confidence intervals of the HC05 for the triangular distribution (**Table 11**).

**Table 11. HC05 and HC50 values (in µg a.i./L) for triangular distribution based on freshwater insect EC/LC50 values.**

|  |  |  |
| --- | --- | --- |
| Distribution | HC05 (95% CI) | HC50 (95% CI) |
| Triangular | 1.10 (0.54-4.40) | 50.6 (23.0-108.4) |



**Figure 4. Triangular SSD for imidacloprid toxicity values for freshwater aquatic insects.**

## Freshwater non-insects

**Figure 5** and **Figure 6** depict the triangular and normal distribution fit to the EC/LC50 values for freshwater non-insect species. **Table 12** includes the HC05 and HC50 values for these two distributions, along with the associated 95% confidence intervals. The triangular distribution, while having the highest AICc weight it not a good visual fit for the data near the HC05 nor HC50. As depicted in **Figure 5** and **Figure 6** the lowest available toxicity value (i.e., 14.2 ug a.i./L for *Gammarus roeseli;* ECOTOX # 160124) is less than all estimated HC05 values.The lowest available toxicity value is however within the 95% confidence intervals of the HC05 for both distributions (**Table 12)**. Therefore, the normal distribution is selected as the best fit for the non-insect invertebrate data.

**Table 12. HC05 and HC50 values (in µg a.i./L) for triangular distribution based on freshwater insect EC/LC50 values.**

|  |  |  |
| --- | --- | --- |
| Distribution | HC05 (95% CI) | HC50 (95% CI) |
| Triangular | 32.4 (11.4-301.8) | 1568 (473-5348) |
| Normal | 26.1 (4.0-234.4) | 1595 (403-6096) |



**Figure 5. Triangular SSD for imidacloprid toxicity values for freshwater aquatic non-insects.**



**Figure 6. Normal SSD for imidacloprid toxicity values for freshwater aquatic non-insects.**

## Comparison of SSDs for aquatic invertebrates and insects

The triangular SSDs for all freshwater aquatic invertebrates and for freshwater aquatic insects were analyzed to determine whether there was a difference in the distributions. **Figure 7** demonstrates that there is little variation between the two distributions around the HC05. However, variation between the two SSD distributions did increase at higher percentiles along the curves.



**Figure 7. Comparison of triangular SSDs for imidacloprid toxicity values for all freshwater aquatic invertebrates and insects.**

## Saltwater invertebrates

**Figure 8** through **Figure 11** depict the four distributions fit to the EC/LC50 values for saltwater invertebrate species (and genera). **Table 13** includes the HC05 and HC50 values for all 4 distributions, along with the associated 95% confidence intervals. When comparing the four distributions to the individual toxicity data, the triangular distribution is not a good visual fit for the data (**Figure 9**).

As depicted in the four figures, the lowest available toxicity value (i.e., 13 ug a.i./L for *Aedes taeniorhynchus;* ECOTOX # 19639) appears to be close to or greater than the estimated HC05 values. The lowest toxicity value from the open literature is however within the 95% confidence intervals of the HC05 for all four distributions (**Table 13**). The gumbel distribution is chosen because the HC05 is conservative while having the best fit to the data, a tight confidence interval, and the highest AICc weight.

**Table 13. HC05 and HC50 values (in µg a.i./L) for gumbel, logistic, normal and triangular distributions based on saltwater invertebrate EC/LC50 values.**

|  |  |  |
| --- | --- | --- |
| Distribution | HC05 (95% CI) | HC50 (95% CI) |
| Gumbel | 11.0 (2.21-154.9) | 459.7 (71.2-4545) |
| Triangular | 6.04 (1.09-312.3) | 1107 (137.2-9140) |
| Normal | 2.88 (0.11-181.6) | 886.8 (80.3-10690) |
| Logistic | 1.41 (0.02-100.4) | 573.3 (46.8-6912) |



**Figure 8. Gumbel SSD for imidacloprid toxicity values for saltwater invertebrates.**



**Figure 9. Triangular SSD for imidacloprid toxicity values for saltwater invertebrates.**



**Figure 10. Normal SSD for imidacloprid toxicity values for saltwater invertebrates.**



**Figure 11. Logistic SSD for imidacloprid toxicity values for saltwater invertebrates.**

# **Conclusions**

For all aquatic invertebrates and for aquatic insects, the normal distribution provided the best fit for the datasets. This decision was based on the AICc weight, confidence limits for the different distributions (especially around the HC05 and HC50) and by visually examining the distributions and their consistency with the toxicity data. The comparison of mortality endpoints (**Table 1**) and median lethal or effects (immobility) concentrations for aquatic invertebrates and insects, demonstrate that there is little variation between the two distributions. Therefore, the triangular distribution for all aquatic invertebrates was determined to be the best fit for the imidacloprid toxicity dataset, and HC05/HC50 from this distribution are used as endpoints for assessing direct effects to all listed freshwater aquatic invertebrates and effects to aquatic invertebrates associated wth PPHD. The gumbel distribution for saltwater aquatic invertebrates provided the best fit for the data and will be used for assessing direct effects to listed estuarine and marine aquatic invertebrates and effects to these invertebrates associated wth PPHD in each category.

1. 24 through 96 hr tests for saltwater invertebrates [↑](#footnote-ref-2)