**APPENDIX 2-5. Clothianidin Species Sensitivity Distribution Analysis for Aquatic Insects**

# **Summary**

Species Sensitivity Distributions (SSDs) were fit to median lethal or effects (immobility) concentrations (LC50 or EC50 values) for aquatic insects exposed to clothianidin. In previous biological evaluations for other insecticides, a combined SSD was conducted for all aquatic invertebrates; however, due to the differences in sensitivity between insects and non-insects, this was not done for clothianidin. Additionally, a separate SSD for mollusks was also developed in previous biological evaluations; however, this was not done for clothianidin due to a lack of data.

Six distributions (normal, logistic, triangular, gumbel, weibull and burr) were fit to the available toxicity data for aquatic insects. For aquatic insects, the triangular distribution provided the best fit for the dataset (**Figure 1**). This decision was based on the AICc weight and confidence limits for the different distributions (especially around the HC05 and HC50). Summary statistics from the fitted SSD for aquatic insects are provided 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).

Diagram

Description automatically generated

**Figure 1. Triangular SSD for clothianidin toxicity values for aquatic insects.**

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

|  |  |
| --- | --- |
| Statistic | Aquatic Insects |
| HC05 (95% CI) | 3.58 (1.42-24-65) |
| HC50 (95% CI) | 176.92 (62.81-500.52) |
| Slope | 1.69 |

CI = confidence interval

# **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, with a minimum of five concentrations of technical grade active ingredient, plus appropriate controls, tested within each study. Additionally, if a definitive immobility and a mortality endpoint were 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. Those data are included in **Table 2**. There was a total of 17 aquatic insect species tested (**Table 3**). 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 unless noted otherwise. For all species, there are one to three different toxicity endpoints (LC50 or EC50 values) available. In cases where two to three endpoints were available for the same test species, values were similar for *Chironomus dilutus* (differing by only 6x), with more variation between the endpoints for *Hexagenia* sp. which differed by 360x. The variation in the endpoints for the two *Hexagenia* sp. was examined and it was noted that ECOTOX #178290 used an immobility-based EC50 and lab cultured organisms, whereas ECOTOX #183503 used a mortality-based LC50 and wild caught organisms. Additionally, since both studies only identified the organisms to genus, it is possible that two different species were used. Therefore, the *Hexagenia* sp. endpoints will be considered as two separate species for this analysis. Using the available data, the slope was calculated for aquatic insects resulting in a median slope of 1.69. The data in **Table 2** are from 8 different studies, with one study (Raby et al., 2018, ECOTOX #178290)[[1]](#footnote-1) representing toxicity data for 12 different test species.

**Table 2. Summary of clothianidin mortality endpoints for aquatic invertebrates (values in µg a.i./L).**

| **Genus or Species** | **Acute EC/LC50 value (µg/L)** | **Slope** | **Reference (ECOTOX #)** |
| --- | --- | --- | --- |
| *Chironomus dilutus* | 2.32 | NA | 173368 |
| *Neocloeon triangulifer* | 3.54 | NA | 178290 |
| *Cheumatopsyche brevilineata* | 4.441 | NA | 152279 |
| *Hexagenia* sp. 1 | 5.51 | NA | 178290 |
| *Chironomus dilutus* | 5.93 | NA | 183458 |
| *Chironomus dilutus* | 13.7 | 0.996 | 178290 |
| *Culex pipiens* ssp. *pipiens* | 14.85 | NA | 183409 |
| *Chironomus riparius* | 221 | NA | MRID 45422414 |
| *Trichocorixa* sp. | 35.4 | 1.62 | 178290 |
| *Gyrinus* sp. | 70.6 | 4.49 | 178290 |
| *Stenelmis* sp. | 224 | 2.98 | 178290 |
| *Aedes aegypti* | 241 | NA | 168249 |
| *Ephemerella* sp. | 668 | 0.975 | 178290 |
| *Cheumatopsyche* sp. | 1470 | 1.76 | 178290 |
| *Maccaffertium* sp. | 1540 | NA | 178290 |
| *Agnetina, Paragnetina* sp. | 1830 | NA | 178290 |
| *Hexagenia* sp. 2 | 2000 | NA | 183503 |
| *Cloeon* sp. | 4570 | 1.18 | 178290 |
| *Coenagrion* sp. | 15500 | 2.08 | 178290 |

NA = Not available

1EC50 value

**Table 3. Distribution of test results available for clothianidin.**

|  |  |  |
| --- | --- | --- |
| Media | Test results | Species |
| Aquatic Insects | 19 | 17 |

# **Determining distribution with best fit**

## P-values

Six potential distributions for the clothianidin 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 clothianidin data and bootstrap goodness-of-fit tests were run with 10,000 replicates. The results of these fitting exercises are presented in **Table 4**. For the aquatic insect SSD, none of the p-values are <0.05, suggesting that none of the distributions should be rejected[[2]](#footnote-2).

**Table 4. P-values calculated for SSDs using aquatic insect toxicity data for clothianidin.**

|  |  |  |
| --- | --- | --- |
| Distribution |  | Aquatic insect SSD |
| Normal |  | 0.14 |
| Logistic |  | 0.08 |
| Triangular |  | 0.19 |
| Gumbel |  | 0.10 |
| Weibull |  | 0.26 |
| Burr |  | 0.07 |

## Akaike’s Information Criteria Weights

Akaike’s Information Criterion corrected for sample size (AICc) was used to compare the six distributions for aquatic insects at the HC052. For aquatic insects, the majority of the weight is attributed to the triangular distribution (with ≤20% each attributed to normal, gumbel, logistic, Weibull and burr; (**Table 5**). Based on the AIC weights, the triangular distribution is used for insects.

**Table 5. Akaike’s Information Criteria (AICc) for distributions for aquatic insect toxicity data for clothianidin.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Distribution | AICc | Delta AICc | Wt | HC05 | SE HC05 |
| Triangular | 254.83 | 0 | 0.41 | 3.58 | 3.08 |
| Normal | 256.28 | 1.44 | 0.20 | 1.91 | 1.89 |
| Gumbel | 256.77 | 1.94 | 0.16 | 3.14 | 2.00 |
| Weibull | 257.43 | 2.60 | 0.11 | 0.44 | 0.73 |
| Logistic | 258.05 | 3.22 | 0.08 | 1.22 | 1.43 |
| Burr | 259.77 | 4.93 | 0.04 | 3.14 | 1.97 |
|  |  |  |  |  |  |

# **Conclusions**

For aquatic insects, the triangular distribution provided the best fit. This decision was based on the AICc weight and confidence limits for the different distributions (especially around the HC05 and HC50). This distribution will be used in the BE to derive HC05 and HC50 values for listed aquatic invertebrates and for assessing effects to PPHD.

1. M. Raby, M. Nowierski, D. Perlov, X. Zhao, C. Hao, D. G. Poirier, and P. K. Sibley. 2018. Acute Toxicity of 6 Neonicotinoid Insecticides to Freshwater Invertebrates. Environmental Toxicology and Chemistry, 37 (5): 1430–1445. ECOTOX# 178290; MRID 50776401 [↑](#footnote-ref-1)
2. Etterson, M. 2011. Appendix C. Analyses of sensitivity distributions for estimation of acute hazard concentrations to aquatic animals. https://www.regulations.gov/document?D=EPA-HQ-OPP-2011-0898-0009 [↑](#footnote-ref-2)