1 Species sensitivity distribution for chlorpyrifos to aquatic organisms: Model

2 choice and sample size

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Abstract

Species sensitivity distribution (SSD) is a widely used model that extrapolates the ecological risk to ecosystem levels from the ecotoxicity of a chemical to individual organisms. However, model choice and sample size significantly affect the development of the SSD model and the estimation of hazardous concentrations at the 5th centile (HC₅). To interpret their effects, the SSD model for chlorpyrifos, a widely used organophosphate pesticide, to aquatic organisms is presented with emphases on model choice and sample size. Three subsets of median effective concentration (EC₅₀) with different sample sizes were obtained from ECOTOX and used to build SSD models based on parametric distribution (normal, logistic, and triangle distribution) and nonparametric bootstrap. The SSD models based on the triangle distribution are superior to the normal and logistic distributions according to several goodness-of-fit techniques. Among all parametric SSD models, the one with the largest sample size based on the triangle distribution gives the most strict HC₅ with 0.141 μmol L⁻¹. The HC₅ derived from the nonparametric bootstrap is 0.159 μmol L⁻¹. The minimum sample size required to build a stable SSD model is 11 based on parametric distribution and 23 based on nonparametric bootstrap. The study suggests that model choice and sample size are important sources of uncertainty for application of the SSD model.

Key words: species sensitivity distribution; aquatic organism; organophosphate pesticide; parametric and nonparametric bootstrap; sample size; model choice

1 Introduction

Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloropyridin-2-pyridinyl phosphorothioate) is one of the most widely used broad spectrum organophosphate pesticides. It acts on the nervous systems of insects by inhibiting acetylcholinesterase (Flaskos, 2012; Giesy et al., 2014). Chlorpyrifos is highly toxic to amphibians and fish and infiltrates aquatic ecosystems via runoff. Because of its long half-life in water, the effects of chlorpyrifos on aquatic ecosystems at different trophic levels are attracting more and more attention (Asselborn et al., 2015; Giddings et al., 2014; Khalil, 2015).

Now, most ecotoxicological data, such as median effective concentration (EC_{50}) or no observed effect concentration (NOEC), are obtained from short-term laboratory experiments on single species at different trophic levels (Vighi et al., 2006). Although they are reproducible, such experiments do not reflect environmental reality. They lack the endpoint and structure that are specific to an ecosystem, and they do not take the dynamic changes of ecosystems into account. Therefore, they lack serious ecological merit (Newman and Unger, 2003). The appearance and application of species sensitivity distribution (SSD) provides an effective way to extrapolate the ecotoxicological endpoint of species to the effects on the ecological community or higher levels (Posthuma et al., 2002).

The SSD model has attracted many researchers and governments to discuss and explore its principles, methods, assumptions, and applications (Newman et

al., 2000). However, it still has some inherent issues, such as model choice, data quantity and quality, and sample size (Chapman et al., 1998; Dowse et al., 2013; Forbes and Forbes, 1993; Hopkin, 1993; Smith and Cairns Jr, 1993; van Straalen, 2002; Wheeler et al., 2002). These issues are the root of uncertainty in SSD models.

Currently, there is no theoretical evidence that SSD belongs to a specific distribution (Forbes and Calow, 2002; Xu et al., 2015). In practice, it is often assumed that the available ecotoxicological data of a chemical follows a certain specific probability distribution such as normal, logistic, triangle, or Burr Type III (Aldenberg and Jaworska, 2000; Aldenberg and Slob, 1993; Larras et al., 2013; Stephan et al., 1985; van Straalen, 2002; Xu et al., 2015). However, a simply chosen probability distribution may not be a good fit for a dataset with a large sample size because of its diverse error source and structure or for a dataset with a small sample size because of the difficulty in estimating the parameters. In such cases, it is necessary to use nonparametric approaches without any assumptions, such as basic bootstrap and modified bootstrap procedures that reduce the uncertainty brought by the model choice (Wang et al., 2008; Xing et al., 2014), but the power of these approaches is also affected by the sample size (Newman et al., 2000).

Representative and valid ecotoxicological data are the basis for a reliable SSD model (Dowse et al., 2013). The assumption of the test species are sampled randomly from the ecosystem, and their response to a specific stress follows a

particular probability distribution (Forbes and Calow, 2002; Posthuma et al., 2002). In fact, they are determined by their availability and are not randomly sampled (Wagner and Løkke, 1991). So far, there is no universal protocol to ensure data quality or to determine the minimum amount of ecotoxicological data for developing an SSD model.

To obtain the hazardous concentrations at the 5th centile (HC₅) of chlorpyrifos and evaluate the effects of model choice and sample size, a series of SSD models is built based on the acute ecotoxicological data (EC₅₀) of chlorpyrifos to aquatic organisms; detailed comparisons are performed among several parametric and nonparametric models with different sample sizes; and, finally, a minimum sample size that is vital to develop a usable SSD model is appraised.

2 Materials and methods

2.1 Data source

From the perspective of reducing uncertainty, EC₅₀ of chlorpyrifos is chosen as the ecotoxicological data upon which to build SSD models. The EC₅₀ of chlorpyrifos (see [Table A](#)) are obtained from ECOTOX, a ecotoxicological database provided by the U.S. Environmental Protection Agency (U.S. Environmental Protection Agency, 2015). To ensure reliability, the EC₅₀ obtained following standardized protocols in the laboratory are chosen. In the case of multiple data points for one species, the geometric mean is used as the estimation of EC₅₀ for

the species (European Commission, 1996; Raimondo et al., 2008; RIVM, 2001).

To quantitatively compare the influence of data sources on an SSD model, three datasets are grouped based on different experimental media and exposure types, namely EC₅₀ from (1) both marine and freshwater media (FW&SW) with the ratio 1:4 for data from marine media to freshwater media, (2) only freshwater media (FW), and (3) only freshwater under static exposure (FW|S).

2.2 Choice and building of SSD model

To evaluate the uncertainty from choosing SSD models, the widely used normal (Aldenberg and Jaworska, 2000), logistic (Aldenberg and Slob, 1993; Larras et al., 2013), and triangle (Stephan et al., 1985) distributions are selected to build the SSD model.

The cumulative distribution function of the logistic distribution is:

$$F(x; \mu, s) = \frac{1}{1 + e^{-\frac{x-\mu}{s}}}$$

where x is the EC₅₀ of chlorpyrifos in logarithmic scale, and μ and s are 2 parameters of the probability distribution function.

The cumulative distribution function of normal distribution is:

$$F(x; \mu, \sigma) = \Phi\left(\frac{x-\mu}{\sigma}\right) = \frac{1}{2} \left[1 + \operatorname{erf}\left(\frac{x-\mu}{\sigma\sqrt{2}}\right) \right]$$

where μ and σ are 2 parameters of the probability distribution function, and erf is the error function.

The cumulative distribution function of the triangle distribution is:

$$F(x; a, b, c) = \begin{cases} 0, & x < a \\ \frac{(x - a)^2}{(b - a)(c - a)}, & a \leq x \leq c \\ 1 - \frac{(b - x)^2}{(b - a)(b - c)}, & c < x \leq b \\ 1, & b < x \end{cases}$$

where a and b are the lower and upper threshold, and c is mode of the probability distribution function.

All parameters are estimated by the maximum likelihood approach (Venables and Ripley, 2002). The confidence interval of each parameter is obtained based on the profile likelihood method. The Kolmogorov-Smirnov and Anderson-Darling tests are used to evaluate the goodness-of-fit (Stephens, 1986). Akaike information criterion (AIC) and Bayesian information criterion (BIC) are used as measures for selecting the optimum SSD model.

2.3 Uncertainty in SSD model and HC₅

To quantitatively address the uncertainty of the SSD model affected by the EC₅₀ of chlorpyrifos, parametric and nonparametric bootstrap are used to generate a simulated dataset (Davison and Hinkley, 1997) on which a corresponding SSD model is fitted with maximum likelihood estimation. The median and confidence interval of each parameter are used to evaluate the uncertainty in the parameters of the SSD model.

To quantitatively measure the uncertainty in HC₅ of chlorpyrifos to aquatic organisms, one nonparametric and three parametric methods are used. For parametric methods, HC₅ is obtained from the SSD model fitting to the normal, logistic, and/or triangle distribution based on (1) the original datasets of EC₅₀,

simulated data with the same sample size generated by (2) parametric bootstrap and (3) by nonparametric bootstrap; and for the nonparametric method, HC_5 is estimated (4) directly from simulated data generated by nonparametric bootstrap. The confidence interval for HC_5 is estimated with the delta method (Oehlert, 1992) for case (1) and the percentile method for the other 3 cases.

2.4 Determination of minimum sample size

To determine the minimum sample size, the HC_5 s based on a series of simulated samples with different size are estimated. The procedure is described as follows: For a given sample size, 5001 samples are generated from the parent sample using random sampling with replacement (Davison and Hinkley, 1997). Based on those samples, the HC_5 and its confidence interval can be estimated according to Section 2.3. This process is replicated for each sample size from 2 to N , the sample size of the parent sample.

The minimum sample size is detected by change point analysis on HC_5 obtained with above procedure. A change point analysis is used to determine a tipping point that separates the response variable (HC_5 in this study) into two groups, each with distinct characteristics, such as the mean and the variance (Qian et al., 2003). The tipping point, which corresponds to the minimum sample size that is the lowest requirement of the ecotoxicological data points for building a stable SSD model, is detected based on the variance changing under a significance level at 0.05.

All simulations and statistical analyses are performed with R (Version 3.11)

(R Core Team, 2014), a language and environment for statistical computing, and add-on packages for R such as *fitdistrplus* (Delignette-Muller and Dutang, 2015), *triangle* (Carnell, 2013), and *ADGofTest* (Bellosta, 2011) for building SSD models and evaluating HC₅ as well as *change point* (Killick and Eckley, 2014) for detecting minimum sample size.

3 Results

3.1 Acute ecotoxicity of chlorpyrifos

207 acute ecotoxicological data (EC₅₀) of chlorpyrifos to seawater and freshwater organisms (FW&SW; see Appendix A) under standardized duration in laboratory with various types of exposure is collected from ECOTOX. The dataset includes 167 data points from FW, and 144 data points from FW|S. The aquatic organisms include amphibians, annelids, crustaceans, fish, insects, marine benthic communities, molluscs, phytoplankton, and zooplankton at various trophic levels. In addition, the dataset also includes part of the standard test organisms such as *Pimephales promelas* and *Daphnia magna*, US endangered species (e.g., *Oncorhynchus mykiss*), and some exotic species such as *Gambusia affinis* and *Eriocheir sinensis*.

3.2 choice of parametric model

All 3 datasets are well fitted with the normal, logistic, and triangle distributions by maximum likelihood estimation. The goodness-of-fit evaluated

by the Kolmogorov-Smirnov and Anderson-Darling tests (Table 1) shows the 3 distributions above can be used to fit the SSD model. It also presents that the triangle distribution has the best goodness-of-fit. In addition, the criteria for model selection (i.e., AIC and BIC) also imply that the triangle distribution is superior to the other two distributions (Table 1). The fitness of the normal and logistic distributions are similar.

Table 1 Goodness-of-fit and criteria for model selection for SSD models based on 3 datasets fitting to 3 probability distributions

Data set	Distribution	Kolmogorov-Smirnov	Anderson-Darling	AIC	BIC
FW&SW (n=207)	Normal	0.084 (0.111)	2.280 (0.065)	709.9	716.5
	Logistic	0.076 (0.187)	2.386 (0.057)	722.9	729.6
	Triangle	0.050 (0.688)	0.505 (0.742)	684.9	694.8
FW (n=167)	Normal	0.080 (0.231)	1.967 (0.096)	576.1	582.3
	Logistic	0.078 (0.259)	1.961 (0.097)	586.0	592.2
	Triangle	0.050 (0.803)	0.427 (0.822)	555.0	564.3
FW S (n=144)	Normal	0.089 (0.204)	1.823 (0.115)	493.5	499.5
	Logistic	0.073 (0.423)	1.767 (0.129)	500.9	506.8
	Triangle	0.065 (0.586)	0.476 (0.771)	476.4	485.3

Note: The number in parentheses is the p -value of the corresponding statistic.

The SSD models fitted to the FW&SW dataset was based on 3 different

195 probability distributions that also were checked by plots of empirical versus
196 theoretical density (Fig. 1a), cumulative density (Fig. 1b) and quantile (Fig. 1c).
197 They clearly show that the triangle distribution fit all data points well; the
198 probability density of the triangle distribution also fits well with the skew of EC_{50}
199 showed by the histogram, which is better than normal, and the logistic
200 distribution, which has a symmetrical density curve. Furthermore, the normal
201 and logistic distributions do not give a reasonable fit at the lower and upper tail
202 of the EC_{50} . The EC_{50} at the low tail is vitally important to the application of the
203 SSD model as HC_5 is sensitive to the fitness of the low tail.

Fig. 1: The empirical-theoretical (a) density-histogram, (b) cumulative density curve, and (c) quantile-quantile (Q-Q) plot of SSD models for FW&SW dataset based on 3 probability distributions.

3.3 SSD model based on the triangle distribution

The parameters of SSD models based on the triangle distribution for FW&SW, FW, and FW|S are estimated by the maximum likelihood method and are close to each other (Fig. 2). The smallest a appears in the FW&SW dataset, and the FW|S dataset has a maximum b . As for the confidence interval of each parameter in different datasets, the narrowest confidence intervals for all 3 parameters appear in the FW&SW dataset while the FW|S has the widest confidence intervals for a and b .

To assess the uncertainty of SSD models based on the triangle distribution, a series of simulated datasets generated by parametric and nonparametric bootstrap based on FW&SW, FW, and FW|S datasets are used. The simulated datasets are also well fitted by the triangle distribution. The parameters, distributions, and confidence intervals are also shown in Fig. 2. The confidence interval for parameters based on simulated data generated by parametric bootstrap is narrower than that based on nonparametric bootstrap. For example, the lower end point at the confidence interval of parameter b exceeds the lower range of the boxplot, although the parameters from both datasets have a similar median (Fig. 2).

All parameters of the triangle distribution for the 3 datasets estimated by the maximum likelihood method are inside the confidence intervals based on the simulated datasets generated by parametric and nonparametric bootstrap (Fig. 2), which suggests that the SSD models of the 3 datasets based on the

triangle distribution are less affected by data selection. The confidence interval of each parameter obtained from original EC₅₀ and from simulated datasets are similar (Fig. 2), thus the SSD model based on the triangle distribution is robust.

Fig. 2: The parameters (a , b , and c of the triangle distribution), their distributions, and confidence intervals of the SSD models are based on the original EC₅₀ datasets, and simulated ones are generated by parametric and nonparametric bootstrap.

MLE means maximum likelihood estimation. P-B MLE and NP-B MLE means performing MLE on the simulated data generation by parametric and nonparametric bootstrap.

Based on the parameters of the SSD model, the SSD curve, which visually represents the sensitivity distribution of different aquatic organisms to chlorpyrifos, can be obtained (Fig. 3). The species in the lower tail of the SSD curve (cumulative probability of 0.05 or less) are 10, 7, and 6 for FW&SW, FW,

and FW|S, respectively. The majority of those species are zooplankton and insects that are highly sensitive to chlorpyrifos. In the FW&SW dataset, *Opsanus beta* is the most sensitive species followed by *Sander vitreus*, *Culicoides variipennis*, and others. In the FW dataset, the most sensitive species is *Poecilia reticulata* followed by *Carassius auratus*, *Thamnocephalus platyurus*, and others. In the FW|S dataset, the most sensitive species is *Thamnocephalus platyurus* followed by *Daphnia magna*, *Ceriodaphnia dubia*, and others. As shown in the SSD curve of the FW&SSD dataset (Fig. 3), the standard test organisms in the lower tail are mainly from *crustaceans*, while threatened/endangered and exotic/nuisance species are in the upper tail. Most of the standard test organisms, especially fish, show a relative tolerance for chlorpyrifos.

Fig. 3: The SSD curve of the FW&SW dataset based on the triangle distribution.

The solid line is the SSD curve fitted by the maximum likelihood estimation. The dotted line

is the 95% confidence interval of the SSD curve based on the profile likelihood method. The shadow area is the confidence interval based on simulated data generated by parametric bootstrap. Only the standard test, threatened/endangered, and exotic/nuisance species are shown in the figure.

Although the goodness-of-fit of the SSD models fitting to the logistic and normal distributions for the 3 datasets are inferior to those of the triangle distribution (Table 1), the parameters estimated by the maximum likelihood method and based on the simulated data generated by parametric and nonparametric bootstrap (results are not shown) show that the SSD models based on both distributions also have good stability.

3.4 HC₅ of chlorpyrifos to aquatic organisms

Based on the parameters of the SSD model built on the triangle distribution, HC₅ and its confidence interval could be calculated for chlorpyrifos to aquatic organisms in FW&SW, FW, and FW|S datasets (Fig. 4). Although HC₅ increases with decreasing sample size, the difference is not significant. The confidence intervals of HC₅ also show the same trend. As is the case with the parameters of the SSD model, the validity of HC₅ also is assessed based on the simulated data generated by parametric and nonparametric bootstrap. The HC₅s based on the original EC₅₀ are similar to those based on simulated datasets, and they are inside the confidence interval of the latter (Fig. 4). Therefore, the HC₅, as

estimated by the SSD model and based on the triangle distribution, is reliable.

The HC₅s of SSD models based on the normal and logistic distributions are also presented in Fig. 4. There are remarkable differences among HC₅s based on different probability distributions. Those differences indicate that model choice is one of the important sources of uncertainty.

To eliminate the impact of model choice on HC₅ estimation, the HC₅ and its distribution are directly estimated from nonparametric bootstrap, which does not need to assume the 3 datasets follow a specific probability distribution (Fig. 4). It is clear that the HC₅ based on nonparametric bootstrap is larger than those based on the parametric SSD model. At the same time, all HC₅s and their confidence intervals of the parametric SSD model based on the triangle distribution are inside that of the nonparametric bootstrap. However, the normal and logistic models give more conservative HC₅s and confidence intervals.

Fig. 4: The HC_5 and its confidence interval are estimated from nonparametric bootstrap, and parametric SSD models which are based on the normal, logistic, and triangle distributions for original EC_{50} and simulated data generated by parametric and nonparametric bootstrap.

MLE means maximum likelihood estimation. P-B MLE and NP-B MLE means performing MLE on the simulated data generation by parametric and nonparametric bootstrap. Bootstrap means to estimate the HC_5 directly from nonparametric bootstrap samples.

3.5 Minimum of sample size

The evaluation of HC_5 is one important role of the SSD model. The HC_5 , which is evaluated from the SSD model based on the simulated data generated from FW&SW, FW, and FW|S datasets, changes along with increasing sample size (Fig. 5).

The HC_5 estimation based on the parametric SSD model shows a rapid decrease with increasing sample size (Fig. 5a-c). With the help of change point analysis, when the sample size reaches 11, 11, and 10 for FW&SW, FW, and FW|S, respectively, HC_5 and the variance of HC_5 does not change significantly with the increasing sample size.

The HC_5 estimated directly from nonparametric bootstrap samples also rapidly decrease with increasing sample size (Fig. 5d-f). The change point analysis also shows a similar pattern, i.e., when the sample size reaches 23, 11, and 11 for FW&SW, FW, and FW|S, respectively, HC_5 and the variance of HC_5 does

not change significantly with increasing sample size.

Fig. 5: Variation of HC₅ estimation against sample size based on the parametric SSD (a)-(c) and nonparametric bootstrap (d)-(f).

4. Discussion

4.1 Data source of SSD model

Ecotoxicological data used in the SSD model should theoretically have a statistical or ecological representative (Dowse et al., 2013). However, there are a large number of SSD models that use the ecotoxicological data obtained in the

laboratory with standard test organisms (Wagner and Løkke, 1991). As shown in Fig. 3, most standard test organisms, except *crustaceans*, are in the upper part of the SSD curve, which indicates that those standard test organisms are not sensitive to chlorpyrifos. Therefore, the SSD model based on such data might give too loose HC₅. The combination of EC₅₀ from nonstandard test organisms will provide better support to the ecological diversity (representative), validity, and reality of the SSD model. In this study, 176 of 207 species are not used as standard test species, and a part of those species are the key species in the food chain and endangered species that need special protection.

Although many studies and guides suggest that applications of the SSD model should use the local species and distinguish different habitats (Stephan et al., 1985), doing so would significantly increase the cost of developing the SSD model and limit data availability, at least to some extent. In this study, the EC₅₀ of chlorpyrifos that used to build the SSD models are involved with various species, habitats, and living regions. The comparison of SSD models based on 3 datasets implies that including the species in seawater media and/or different exposure types can increase the number of species, facilitate the stability of SSD models, and produce a more strict HC₅, indicating better protection of the aquatic ecosystem. The SSD model itself does not show significant differences for the 3 datasets. To some extent, due to a larger number of species, the SSD model including more species may have more stability and less uncertainty (Raimondo et al., 2008). Furthermore, the difference in sensitivity between the same species

in different regions is generally far lower than the difference between different species in the same region (Hose and van den Brink, 2004).

4.2 Choice of SSD model

Currently, there is no theoretical study that has implied that the SSD belongs to a certain distribution (Forbes and Calow, 2002; Xu et al., 2015). Therefore, many probability distributions have been used to fit the species sensitivity. Among them, the logistic (Aldenberg and Slob, 1993; Larras et al., 2013), normal (Aldenberg and Jaworska, 2000), and triangle distributions (Stephan et al., 1985) are widely used types of probability distribution. As shown in [Table 1](#), the SSD model based on the triangle distribution has the smallest Anderson-Darling statistic, indicating that the triangle distribution can fit the chlorpyrifos data very well. Besides the goodness-of-fit ranking, which favors more parameters of a specific SSD model, AIC and BIC also indicate that the triangle distribution is a better choice.

The triangle distribution is generally used as a threshold model to fit the SSD model. It has a lower threshold a , which is treated as HC_0 . That means when the stress is lower than it, no species would be affected. Although a threshold for chlorpyrifos in acute tests cannot be expected, the ecotoxicological response of various species to chlorpyrifos should be in a finite set. Furthermore, the histogram of EC_{50} of chlorpyrifos is not symmetrical ([Fig 1a](#)) and has an obvious skew, thus a distribution that could describe a skew variable (e.g., the triangle distribution) also could be a better fit than the normal or logistic distributions

that have a symmetrical probability density curve. As a result, it is reasonable to suggest that the triangle distribution can fit well with the EC₅₀. Van Straalen (2002) also confirmed that the triangle distribution, as a threshold model, could make the best fit for the NOEC of zinc to aquatic organisms among the uniform, triangle, exponential, and Weibull distributions.

Nevertheless, there is no reason to conclude that the sensitivity of aquatic organisms to chlorpyrifos follows the triangle distribution because there are many possible probability distributions that could be chosen including Burr Type III and skew-normal distribution, both of which were tested and fit the datasets used in this study very well (results not shown). But there are significant differences among the HC₅ evaluated from the SSD models based on different probability distributions.

On the other hand, the model-free method, such as basic or modified bootstrap, does not assure the HC₅ evaluation will be accurate, either. It is simply an alternative means to obtain HC₅ that have less uncertainty as a result of the choice of a specific probability distribution.

4.3 Minimum sample size

Generally, the development of the SSD model faces a lack of ecotoxicological data. At the same time, although there are many studies that involve the sample size requirements, there is no universal protocol that establishes the minimum sample size. The methods to evaluate the minimal sample size generally include the basic or modified bootstrap. In this study, both nonparametric bootstrap and

the combination of bootstrap with parametric methods are used. According to the simulation based on the parametric SSD model, at least 10 EC₅₀ are required to build a stable SSD model. That result is consistent with Wheeler et al. (2002) who suggest that 10 or more ecotoxicological data are needed to estimate HC₅. Maltby et al. (2005) suggest at least 6 ecotoxicological data are need when the Anderson-Darling test is used to evaluate the SSD model based on the normal distribution. The OECD requires chronic ecotoxicological data from at least 5 species.

Information on the number of taxonomic groups is not included in the analysis for the minimum sample size. However, taxonomic groups included in the SSD model could give a simple representation of the trophic structure of the ecosystem and provide more ecological context. Thus, it plays an important role in the application of the SSD model, especially when sample sizes are small. Thus, some countries and/or organizations are required to include taxonomic information in SSD models as well as the requirement for a minimal sample size. The EU requires at least 10 chronic ecotoxicological data from 8 different taxonomies to derive the water quality baseline for specific pollutants using the SSD model (European Commission, 1996). The Netherlands requires chronic ecotoxicological data from at least 4 different taxonomies (RIVM, 2001). The US EPA requires acute or chronic ecotoxicological data from at least 3 categories, 8 families of species to derive maximum concentration criteria, and sustained concentration criteria (Stephan et al., 1985).

Newman et al. (2000) suggest that the minimum sample size is in the range of 15 to 55 when building an SSD model based on nonparametric bootstrap. In the present study, the nonparametric bootstrap determined that the minimum sample size of 10 ~ 23 is required to build a stable SSD model. The larger the size of the parent sample, the higher the minimum sample size required. This is largely due to the range and empirical distribution of the parent sample. For the FW&SW dataset, due to the diversity of aquatic organisms and the variation of the corresponding response to chlorpyrifos, the samples generated by the nonparametric bootstrap vary significantly. The HC₅ can reach stability only with a large sample size.

4.4 Uncertainty of the SSD model

The uncertainty of the SSD model has many sources that include the data source, model selection, and sample size (Forbes and Calow, 2002; Xu et al., 2015). In this study, the effects of data sources on model parameters, and HC₅ is characterized through the simulation of parametric and nonparametric bootstrap resampling techniques. Although the 3 SSD models have good robustness, there are still some differences in their parameters (for the triangle distribution, see [Fig. 2](#)). At the same time, the choice of distribution is also compared. The HC₅ from SSD models based on different types of probability distribution show there are differences ([Fig. 4](#)). High HC₅ appears in the SSD model based on the triangle distribution, while the others are low. This implies that the estimated HC₅ may under- or over-protect the ecosystem because of the model chosen.

For the normal and logistic distributions, Aldenberg and Slob (1993) and Aldenberg and Jaworska (2000) simulated the Bayesian uncertainty of parameter estimates and HC₅, which are better compared to confidence intervals, a measure of uncertainty, estimated by ELM. In this study, the widths of the confidence interval from the normal distribution based on parametric bootstrap and Bayesian statistics and that from nonparametric bootstrap are similar; those from the logistic distribution and based on parametric bootstrap are wider; and those based on Bayesian statistics are narrower (results not shown). Because a noninformative prior was used, the parametric bootstrap and Bayesian statistics should give similar results.

Newman et al. (2000) suggest using the nonparametric bootstrap method to build the SSD model because it does not assume the data needed to follow a specific probability distribution, thereby reducing the uncertainty brought by model selection, although model-free does not assure that the HC₅ from a nonparametric bootstrap would have less or no uncertainty. Nonparametric bootstrap is a resampling method based on the original data. By expanding the sample size through resampling, an empirical distribution of statistics could be obtained and used to perform statistical inference (Newman et al., 2000; Verdonck et al., 2001). The HC₅ obtained with nonparametric bootstrap also significantly differs with all 3 distributions. Although it does not make any assumptions on the distribution, the results are also severely affected by the sample size of the parent sample. The small sample size is likely to cause low

resolution (Davison and Hinkley, 1997).

5. Conclusions

In this study, the EC_{50} of chlorpyrifos to aquatic organisms are collected from ECOTOX. Then SSD models based on various probability distributions were developed, and the one based on the triangle distribution showed the best goodness-of-fit. From the perspective of the entire aquatic ecosystem, chlorpyrifos mainly threatens aquatic organisms at the lower trophic level. According to the SSD model of FW&SW, the HC_5 of chlorpyrifos is $0.141\mu\text{mol/L}$ based on the triangle distribution, and $0.159\mu\text{mol/L}$ based on nonparametric bootstrap. This study also shows that the model choice is an important source of uncertainty in the applications of the SSD model to ecological risk assessment.

The sample size has significant impact on the robustness and uncertainties of the SSD model. The simulated SSD model that was based on the parametric and nonparametric bootstrap indicates the number of species to be at least above 10 ~ 23 can be used to build a stable SSD model, and the larger the number of species, the more robust the SSD model.

Although a reliable SSD model could be developed based on the toxicological data of a wide range of aquatic organisms and applied to derivate environmental quality standards or ecological criteria and ecological risk assessment, the methods for analyzing and reducing uncertainty need further study to establish a unified framework and procedures to promote the development and application

of the SSD model.

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619

620 **Appendix**621 **Table A: The aquatic organisms in FW&SW and their EC₅₀ to chlorpyrifos (µg/L)**

No.	Species	Group	Media Type	EC ₅₀
1	<i>Aedes aegypti</i>	Insects/Spiders	FW S	14.455
2	<i>Aedes albopictus</i>	Insects/Spiders*	FW S	393.166
3	<i>Aedes atropalpus</i>	Insects/Spiders	FW S	0.600
4	<i>Aedes canadensis</i>	Insects/Spiders	FW S	0.549
5	<i>Aedes cantans</i>	Insects/Spiders	FW S	1.100
6	<i>Aedes communis</i>	Insects/Spiders	FW S	1.200
7	<i>Aedes excrucians</i>	Insects/Spiders	FW S	3.300
8	<i>Aedes punctor</i>	Insects/Spiders	FW S	2.700
9	<i>Aedes sticticus</i>	Insects/Spiders	FW S	0.500
10	<i>Aedes triseriatus</i>	Insects/Spiders	FW S	0.532
11	<i>Aedes vexans</i>	Insects/Spiders	FW S	0.665
12	<i>Agamermis unka</i>	Worms	FW S	1210.000
13	<i>Ambystoma mexicanum</i>	Amphibians	FW	1360.000
14	<i>Americamysis bahia</i>	Crustaceans+	SW	0.065
15	<i>Ampelisca abdita</i>	Crustaceans+	SW	0.302
16	<i>Amphiascus tenuiremis</i>	Crustaceans	SW	2.844
17	<i>Anax imperator</i>	Insects/Spiders	FW S	3.561
18	<i>Anguilla anguilla</i>	Fish	FW S	783.329
19	<i>Anisops sardeus</i>	Insects/Spiders	FW S	1.192

20	<i>Anopheles albimanus</i>	Insects/Spiders	FW S	8.000
21	<i>Anopheles freeborni</i>	Insects/Spiders	FW S	3.000
22	<i>Anopheles gambiae</i>	Insects/Spiders	FW S	3.108
23	<i>Anopheles quadrimaculatus</i>	Insects/Spiders	FW S	1.000
24	<i>Anopheles sinensis</i>	Insects/Spiders	FW S	4700.000
25	<i>Anopheles stephensi</i>	Insects/Spiders	FW S	1.123
26	<i>Aphanius iberus</i>	Fish	SW	38.600
27	<i>Aquatic community</i>	Miscellaneous	FW S	10.000
28	<i>Artemia parthenogenetica</i>	Crustaceans	SW	558.570
29	<i>Artemia salina</i>	Crustaceans	SW	532.581
30	<i>Artemia sp.</i>	Crustaceans	SW	149.065
31	<i>Asellus aquaticus</i>	Crustaceans	FW S	8.580
32	<i>Atalophlebia australis</i>	Insects/Spiders	FW	0.292
33	<i>Atherinops affinis</i>	Fish+	SW	4.975
34	<i>Aulacomya ater</i>	Molluscs	SW	16.000
35	<i>Belostoma sp.</i>	Insects/Spiders	FW S	15.000
36	<i>Berosus styliferus</i>	Insects/Spiders	FW S	9.000
37	<i>Bidyanus bidyanus</i>	Fish	FW	17.000
38	<i>Brachionus calyciflorus</i>	Invertebrates+	FW S	6665.795
39	<i>Brachionus plicatilis</i>	Invertebrates+	SW	2635.565
40	<i>Branchiura sowerbyi</i>	Worms	FW S	66.000
41	<i>Bufo bufo</i> ssp. gargarizans	Amphibians	FW	1503.353

42	<i>Bulinus truncatus</i>	Molluscs	FW S	1320.000
43	<i>Callinectes sapidus</i>	Crustaceans	SW	11.000
44	<i>Carassius auratus</i>	Fish*	FW	3270.000
45	<i>Ceriodaphnia dubia</i>	Crustaceans+	FW S	0.075
46	<i>Channa punctata</i>	Fish	FW	544.401
47	<i>Chaoborus obscuripes</i>	Insects/Spiders	FW S	1.533
48	<i>Chironomus crassicaudatus</i>	Insects/Spiders	FW S	52.000
49	<i>Chironomus decorus</i>	Insects/Spiders	FW S	21.949
50	<i>Chironomus dilutus</i>	Insects/Spiders	FW S	0.514
51	<i>Chironomus plumosus</i>	Insects/Spiders	FW S	49.630
52	<i>Chironomus riparius</i>	Insects/Spiders+	FW S	2774.000
53	<i>Chironomus salinarius</i>	Insects/Spiders	SW	0.990
54	<i>Chironomus sp.</i>	Insects/Spiders+	FW S	0.600
55	<i>Chironomus tentans</i>	Insects/Spiders+	FW S	3.83
56	<i>Chironomus utahensis</i>	Insects/Spiders	FW S	1.981
57	<i>Chirostoma jordani</i>	Fish	FW S	0.170
58	<i>Chlorella sp.</i>	Algae, Moss, Fungi	FW S	1290.000
59	<i>Cirrhinus mrigala</i>	Fish	FW S	2208.108
60	<i>Claassenia sabulosa</i>	Insects/Spiders	FW S	2.034
61	<i>Clarias gariepinus</i>	Fish	FW S	920.000
62	<i>Cloeon dipterum</i>	Insects/Spiders	FW S	0.627
63	<i>Corixa punctata</i>	Insects/Spiders	FW	2.855

64	<i>Cricotopus sp.</i>	Insects/Spiders	FW S	42.368
65	<i>Crocothemis erythraea</i>	Insects/Spiders	FW S	5.800
66	<i>Culex melanurus</i>	Insects/Spiders	FW S	1.037
67	<i>Culex peus</i>	Insects/Spiders	FW S	1.036
68	<i>Culex pipiens</i>	Insects/Spiders	FW S	13.332
69	<i>Culex pipiens</i> ssp. molestus	Insects/Spiders	FW S	0.736
70	<i>Culex pipiens</i> ssp. pallens	Insects/Spiders	FW S	95.270
71	<i>Culex pipiens</i> ssp. pipiens	Insects/Spiders	FW S	0.666
72	<i>Culex pipiens</i> ssp. quinquefasciata	Insects/Spiders	FW S	1.228
73	<i>Culex quinquefasciatus</i>	Insects/Spiders	FW S	14.948
74	<i>Culex restuans</i>	Insects/Spiders	FW S	0.392
75	<i>Culex tarsalis</i>	Insects/Spiders	FW S	0.530
76	<i>Culex tritaeniorhynchus</i>	Insects/Spiders	FW S	0.320
77	<i>Culicoides sp.</i>	Insects/Spiders	SW	0.500
78	<i>Culicoides variipennis</i>	Insects/Spiders	FW S	3.023
79	<i>Culiseta annulata</i>	Insects/Spiders	FW S	3.500
80	<i>Culiseta incidens</i>	Insects/Spiders	FW S	2.924
81	<i>Cyprinodon variegatus</i>	Fish+	SW	191.625
82	<i>Cyprinus carpio</i>	Fish+*	FW S	23.935
83	<i>Daphnia ambigua</i>	Crustaceans	FW S	0.035
84	<i>Daphnia carinata</i>	Crustaceans	FW S	0.224

85	<i>Daphnia longispina</i>	Crustaceans	FW S	0.490
86	<i>Daphnia magna</i>	Crustaceans+	FW S	1.635
87	<i>Daphnia pulex</i>	Crustaceans+	FW S	0.468
88	<i>Deleatidium sp.</i>	Insects/Spiders	FW S	0.050
89	<i>Diaptomus forbesi</i>	Crustaceans	FW S	3.600
90	<i>Dicrotendipes californicus</i>	Insects/Spiders	FW S	16.733
91	<i>Donax faba</i>	Molluscs	SW	247.72
92	<i>Dunaliella tertiolecta</i>	Algae, Moss, Fungi	SW	769.000
93	<i>Ephemerella sp.</i>	Insects/Spiders	FW	0.400
94	<i>Eriocheir sinensis</i>	Crustaceans*	FW, SW	71.434
95	<i>Fundulus grandis</i>	Fish	SW	1.800
96	<i>Fundulus similis</i>	Fish	SW	4.100
97	<i>Gambusia affinis</i>	Fish*	FW S, SW	327.665
98	<i>Gambusia yucatana</i>	Fish	FW S	11.000
99	<i>Gammarus fasciatus</i>	Crustaceans+*	FW S	1.339
100	<i>Gammarus fossarum</i>	Crustaceans	FW	1.017
101	<i>Gammarus lacustris</i>	Crustaceans+	FW S	0.322
102	<i>Gammarus palustris</i>	Crustaceans	SW	1.179
103	<i>Gammarus pseudolimnaeus</i>	Crustaceans+	FW	0.244
104	<i>Gammarus pulex</i>	Crustaceans	FW S	0.424
105	<i>Gasterosteus aculeatus</i>	Fish+*	FW	10.672
106	<i>Gibelion catla</i>	Fish	FW S	1308.943

107	<i>Globodera rostochiensis</i>	Worms	FW S	1.220
108	<i>Glyptotendipes paripes</i>	Insects/Spiders	FW S	12.000
109	<i>Goeldichironomus holoprasinus</i>	Insects/Spiders	FW S	380.000
110	<i>Heptageniidae</i>	Insects/Spiders	FW S	14.248
111	<i>Heteropneustes fossilis</i>	Fish	FW S	3086.879
112	<i>Hyalella azteca</i>	Crustaceans+	FW S	0.089
113	<i>Hydra attenuate</i>	Invertebrates	SW	1224.745
114	<i>Hydrophilus sp.</i>	Insects/Spiders	FW S	100.000
115	<i>Hydrophilus triangularis</i>	Insects/Spiders	FW S	24.495
116	<i>Hydropsychidae</i>	Insects/Spiders	FW S	30.000
117	<i>Hygrotus sp.</i>	Insects/Spiders	FW S	40.000
118	<i>Ictalurus punctatus</i>	Fish+	FW S	348.818
119	<i>Ischadium recurvum</i>	Molluscs	SW	960.000
120	<i>Isochrysis galbana</i>	Algae, Moss, Fungi	SW	138.000
121	<i>Labeo rohita</i>	Fish	FW S	1595.551
122	<i>Laccophilus maculosus ssp.</i>	Insects/Spiders	FW S	4.600
	Maculosus			
123	<i>Lampsilis siliquoidea</i>	Molluscs	FW S	381.960
124	<i>Lanistes carinatus</i>	Molluscs	FW S	2710.000
125	<i>Lepomis cyanellus</i>	Fish	FW S	74.162
126	<i>Lepomis macrochirus</i>	Fish+	FW S	5.938
127	<i>Leptoceridae</i>	Insects/Spiders	FW S	0.818

128	<i>Leuresthes tenuis</i>	Fish	SW	1.827
129	<i>Litopenaeus vannamei</i>	Crustaceans	SW	4.800
130	<i>Macrobrachium lar</i>	Crustaceans	FW	0.961
131	<i>Maja squinado</i>	Crustaceans	SW	0.815
132	<i>Maxillopoda</i>	Crustaceans	FW S	2.130
133	<i>Melanotaenia fluviatilis</i>	Fish	FW	229.676
134	<i>Menidia beryllina</i>	Fish+	SW	6.545
135	<i>Menidia menidia</i>	Fish+	SW	2.879
136	<i>Menidia peninsulae</i>	Fish+	SW	1.328
137	<i>Moina australiensis</i>	Crustaceans	FW S	0.126
138	<i>Morone saxatilis</i>	Fish	SW	0.580
139	<i>Mugil cephalus</i>	Fish	SW	5.400
140	<i>Neocaridina denticulata</i>	Crustaceans	FW S	692.913
141	<i>Neomysis integer</i>	Crustaceans	SW	0.155
142	<i>Neoplea striola</i>	Insects/Spiders	FW S	1.955
143	<i>Nitzschia sp.</i>	Algae, Moss, Fungi	FW S	2215.349
144	<i>Notonecta maculata</i>	Insects/Spiders	FW S	13.802
145	<i>Odonata</i>	Insects/Spiders	FW S	11.400
146	<i>Oncorhynchus clarkii</i>	Fish	FW S	27.126
147	<i>Oncorhynchus mykiss</i>	Fish+*	FW S	27.100
148	<i>Opsanus beta</i>	Fish	SW	188.043
149	<i>Orconectes immunis</i>	Crustaceans	FW	6.000

150	<i>Oreochromis mossambicus</i>	Fish	FW S	26.608
151	<i>Oreochromis niloticus</i>	Fish	FW S	89.096
152	<i>Oryzias latipes</i>	Fish+	FW S	91.387
153	<i>Oscillatoria sp.</i>	Algae, Moss, Fungi	FW S	330.000
154	<i>Oziotelphusa senex</i> ssp. senex	Crustaceans	FW S	584.818
155	<i>Palaemon macrodactylus</i>	Crustaceans	SW	0.050
156	<i>Palaemon serratus</i>	Crustaceans	SW	0.277
157	<i>Palaemonetes pugio</i>	Crustaceans	SW, FW	1.911
158	<i>Paramelita nigroculus</i>	Crustaceans	FW S	0.900
159	<i>Parapoynx stratiotata</i>	Insects/Spiders	FW S	35.320
160	<i>Paratya australiensis</i>	Crustaceans	FW	0.216
161	<i>Peltodytes sp.</i>	Insects/Spiders	FW S	0.800
162	<i>Penaeus monodon</i>	Crustaceans	SW	27.612
163	<i>Pimephales promelas</i>	Fish+	FW S	194.336
164	<i>Plea minutissima</i>	Insects/Spiders	FW S	5.088
165	<i>Poecilia reticulata</i>	Fish+	FW S	47.279
166	<i>Pomacea canaliculata</i>	Molluscs*	FW S	978.000
167	<i>Procambarus acutus</i> ssp. acutus	Crustaceans	FW S	2.000
168	<i>Procambarus clarkii</i>	Crustaceans	FW	26.145
169	<i>Procambarus sp.</i>	Crustaceans	FW S	8.381
170	<i>Procladius sp.</i>	Insects/Spiders	FW S	2.609
171	<i>Procloeon sp.</i>	Insects/Spiders	FW	0.081

172	<i>Psetta maxima</i>	Fish	SW	116.6
173	<i>Pseudacris regilla</i>	Amphibians	FW S	121.87
174	<i>Pseudagrion sp.</i>	Insects/Spiders	FW S	0.100
175	<i>Pteronarcella badia</i>	Insects/Spiders	FW S	1.422
176	<i>Pteronarcys californica</i>	Insects/Spiders	FW S	20.801
177	<i>Pungitius pungitius</i>	Fish	FW	5.176
178	<i>Rana boylei</i>	Amphibians	FW S	785.332
179	<i>Rana dalmatina</i>	Amphibians	FW S	5174.000
180	<i>Ranatra linearis</i>	Insects/Spiders	FW S	10.646
181	<i>Rhepoxynius abronius</i>	Crustaceans+	SW	0.099
182	<i>Rhinella arenarum</i>	Amphibians	FW S	23300
183	<i>Rhinella fernandezae</i>	Amphibians	FW	482.431
184	<i>Romanomermis culicivorax</i>	Worms	FW S	2.100
185	<i>Salvelinus namaycush</i>	Fish+	FW S	185.847
186	<i>Sander vitreus</i>	Fish	FW S	41.255
187	<i>Sialis lutaria</i>	Insects/Spiders	FW S	21700.000
188	<i>Sigara arguta</i>	Insects/Spiders	FW S	2.160
189	<i>Simocephalus vetulus</i>	Crustaceans	FW S	0.271
190	<i>Simulium vittatum</i>	Insects/Spiders	FW S	0.455
191	<i>Skeletonema costatum</i>	Algae, Moss, Fungi+	SW	375.616
192	<i>Spiralothelphusa hydrodroma</i>	Crustaceans	FW	156.891
193	<i>Streptocephalus sudanicus</i>	Crustaceans	FW S	5.358

194	<i>Tanytarsus sp.</i>	Insects/Spiders	FW S	20.785
195	<i>Thalassiosira pseudonana</i>	Algae, Moss, Fungi	SW	148.000
196	<i>Thamnocephalus platyurus</i>	Crustaceans	FW S	0.530
197	<i>Thermonectus basillaris</i>	Insects/Spiders	FW S	6.000
198	<i>Tilapia aurea</i>	Fish*	FW S	418.000
199	<i>Tilapia guineensis</i>	Fish	SW	2.000
200	<i>Tilapia sp.</i>	Fish	FW	1774.000
201	<i>Tilapia zillii</i>	Fish	FW S	73.876
202	<i>Trichodactylus borellianus</i>	Crustaceans	FW S	242.320
203	<i>Triops longicaudatus</i>	Crustaceans	FW S	4.000
204	<i>Tropisternus lateralis</i>	Insects/Spiders	FW S	20.396
205	<i>Xanthocnemis zealandica</i>	Insects/Spiders	FW S	8.440
206	<i>Xenopus laevis</i>	Amphibians+	FW S	2767.184
207	<i>Zacco platypus</i>	Fish	FW S	1700.000

622 Note: * is US Threatened and Endangered Species or U.S. Exotic/Nuisance Species

623 + is Standard Test Species

624 SW, FW, and FW|S represent seawater, freshwater, and freshwater with static condition.