

Advancing Fifth Percentile Hazard Concentration Estimation Using Toxicity-Normalized Species Sensitivity Distributions

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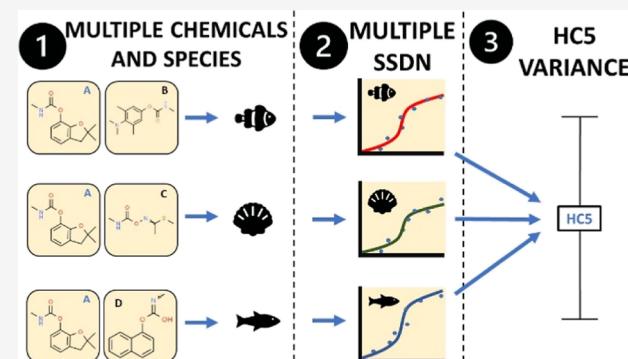
Supporting Information

ABSTRACT: The species sensitivity distribution (SSD) is an internationally accepted approach to hazard estimation using the probability distribution of toxicity values that is representative of the sensitivity of a group of species to a chemical. Application of SSDs in ecological risk assessment has been limited by insufficient taxonomic diversity of species to estimate a statistically robust fifth percentile hazard concentration (HC₅). We used the toxicity-normalized SSD (SSD_n) approach, (Lambert, F. N.; Raimondo, S.; Barron, M. G. *Environ. Sci. Technol.* 2022, 56, 8278–8289), modified to include all possible normalizing species, to estimate HC₅ values for acute toxicity data for groups of carbamate and organophosphorous insecticides. We computed mean and variance of single chemical HC₅ values for each chemical using leave-one-out (LOO) variance estimation and compared them to SSD_n and conventionally estimated HC₅ values. SSD_n-estimated HC₅ values showed low uncertainty and high accuracy compared to single-chemical SSDs when including all possible combinations of normalizing species within the chemical-taxa grouping (carbamate-all species, carbamate-fish, organophosphate-fish, and organophosphate-invertebrate). The SSD_n approach is recommended for estimating HC₅ values for compounds with insufficient species diversity for HC₅ computation or high uncertainty in estimated single-chemical HC₅ values. Furthermore, the LOO variance approach provides SSD practitioners with a simple computational method to estimate confidence intervals around an HC₅ estimate that is nearly identical to the conventionally estimated HC₅.

KEYWORDS: acetylcholinesterase inhibitor, new approach method, species sensitivity distribution, hazard concentration

1. INTRODUCTION

The species sensitivity distribution (SSD) is an internationally accepted approach to hazard estimation using the probability distribution of toxicity values that is representative of the sensitivity of a group of species to a chemical.^{2–4} The fifth percentile of the SSD, defined as the hazard concentration for 5% of the species (HC₅), has been frequently used in deriving water quality standards for aquatic life. However, application of SSDs in ecological risk assessment and the development of water quality standards has been limited by several factors. First, there is no consensus among regulatory bodies on the minimum taxonomic data requirements needed to generate a robust SSD. Guidance for US water quality criteria mandates a minimum sample size of eight toxicity tests from eight distinct taxonomic groups;⁵ however, the European Chemicals Agency mandates a minimum of 10 tests from eight distinct taxonomic groups⁶ and, more recently, Carr and Belanger⁷ have suggested a minimum sample size of 13. Second, the taxonomic composition of the SSD can have a greater influence on HC₅ estimates than the number of species for those chemical groups with substantial taxa-specific differences in sensitivity such as insecticides.⁸ Finally, statistical issues with model fitting⁴ and obtaining



sufficient toxicity data under various guidelines have been major hindrances to SSD development.

Conventionally, SSDs have been derived from experimentally determined toxicity data for a single chemical, and the derived HC₅ values are chemical-specific. Computational advances have allowed for filling toxicity data gaps in species number and diversity using predictive modeling and leveraging data for groups of toxicologically similar chemicals (e.g., Coleman and Edmands⁹ and Willming et al.¹⁰). Recently, Giddings et al.⁸ developed the toxicity-normalized SSD (SSD_n) approach, a grouped chemical method that creates a single combined SSD_n by normalizing toxicity data using a species tested in all members of the chemical group (a normalization species or nSpecies). Chemical-specific HC₅s are then back-calculated from the SSD_n-estimated HC₅ and the chemical-specific toxicity value of

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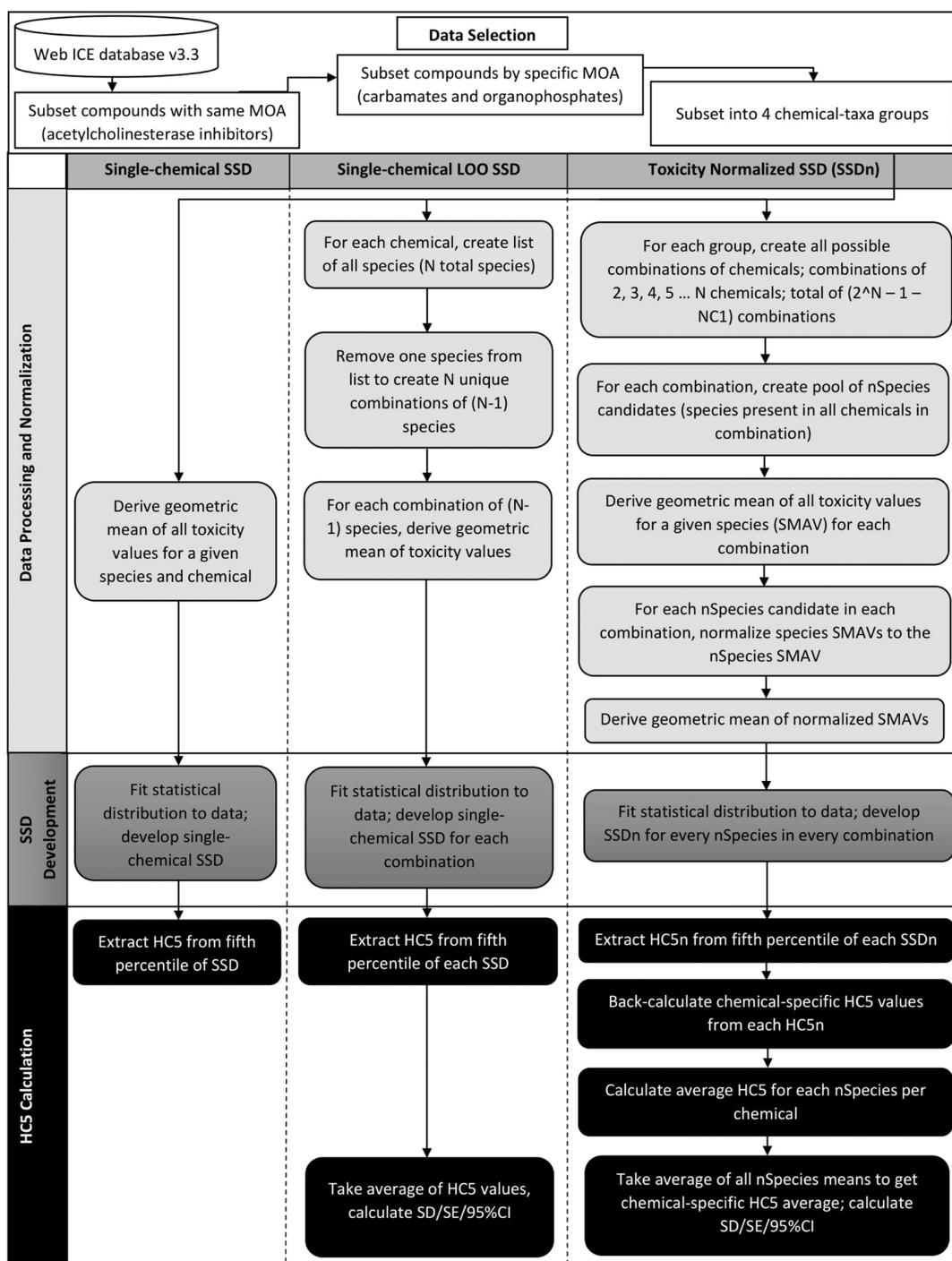


Figure 1. Flow diagram of SSD generation using conventional, LOO, and SSDn approaches.

the nSpecies. Lambert et al.¹ extended the SSDn method using a case study of heavy metals and provided guidelines for computing HCS estimates for chemicals that lack adequate taxonomic representation in conventional single-chemical SSDs.

Despite advantages of leveraging toxicity data across a group of chemicals to increase taxonomic diversity and species number, a key limitation of the SSDn approach has been the requirement of a single nSpecies tested in all compounds within the chemical group.^{1,8} To address this limitation in developing a pyrethroid insecticide SSDn, Giddings et al.⁸ conducted supplemental toxicity tests on the nSpecies (*Hyalella azteca*) so that all chemicals in the group shared a common test species.

In addition to the single nSpecies (*Daphnia magna*) used in the grouping of nine heavy metals, Lambert et al.¹ used alternative normalizing species and smaller chemical groups in determining that the relative sensitivity of the normalizing species and size of the chemical group had little influence on the accuracy of SSDn-estimated HCSs.

The goal of the current study was to advance HCS estimation using the SSDn approach by addressing three objectives using a highly curated dataset of acute aquatic toxicity values. The first objective was to compute mean and variance of HCS values estimated from conventional single-chemical SSDs to allow comparison to SSDn-estimated HCSs. The second objective was

Table 1. Chemical and Taxa Groupings of ACHEI Used in Computing SSDs, Number of Species in Each Chemical-Specific SSD, and Ranges in LC₅₀ and HCS Values^a

ACHEI group	taxa subgroup	chemicals in group	species per chemical	LC ₅₀ (μg/L)	HCS (μg/L) ^b
carbamate	all species	5	13–50	1.70–31,000	2.78–81.7
	fish	5	9–35	33.0–31,000	51.9–6630
organophosphate	invertebrate	10	8–19	0.035–301,000	0.000653 ^c –0.250
	fish	13	8–21	0.400–110,000	0.224–1410

^aA more detailed table can be found in Supporting Information Table S1. ^bEstimated from conventional single-chemical SSDs. ^cRange with dichlorvos outlier. Range with censored dichlorvos HCS: 0.01–0.250 μg/L.

to extend the SSDn method to organophosphate and carbamate insecticides, a class of compounds with a mode of action (MOA) of acetylcholinesterase inhibition (ACHEI) and two distinct structural subgroups. The ACHEIs have an existing large dataset of highly curated acute toxicity values with high species diversity, distinct sensitivity between aquatic vertebrate and invertebrate taxa, and orders of magnitude ranges in toxicity values among taxa. The third objective was to develop a method to calculate the central tendency and variance in SSDn-estimated HCS values and to assess the influence of using multiple nSpecies on the accuracy and uncertainty of the approach. The incorporation of all available nSpecies builds upon the original SSDn work of Giddings et al.⁸ and Lambert et al.¹ and advances the SSD approach based on the foundational work of Stephan et al.⁵ and others.

2. METHODS

2.1. Experimental Overview. The process used in the current study is outlined as a flowchart (Figure 1) and included five discrete steps: (1) dataset compilation and subgrouping, (2) generation of conventional single-chemical SSDs and HCS estimation, (3) computation of single-chemical HCS values and 95% confidence intervals using LOO mean and variance estimation, (4) generation of SSDn-estimated HCS values and calculation of mean and variance using all available nSpecies, and (5) a meta-analysis and validation of the SSDn ACHEI results. The final ACHEI dataset (SI-Data), R files used in the analysis (SI-Code), a glossary of terms used in the SSDn approach (Table S2), a censoring analysis (SI-Censoring), and user manual detailing the methodology (SI-Manual) are provided in the Supporting Information.

2.2. Dataset Compilation and Subgrouping. Acute aquatic toxicity data (48 and 96 h LC₅₀ and EC₅₀ values) for fish, invertebrates, and amphibians were compiled from the Web-ICE database¹¹ (v3.3; <https://www3.epa.gov/webice/>). The Web-ICE database contains acute toxicity data including both nominal and analytically confirmed concentrations; however, it is extensively curated and standardized through inspection of primary literature sources and adherence to specific quality control guidelines.¹⁰ The Web-ICE database also contains MOA and taxonomic assignments. Only chemicals that were classified with an MOA of acetylcholinesterase inhibitors and a subcategory of either organophosphate or carbamate were selected. These data were filtered to contain only chemicals with toxicity values for a minimum of eight per species when subgrouped by the ACHEI subcategory (carbamate and organophosphate) and taxa composition (fish, aquatic invertebrate, and all aquatic species). A minimum of eight species were chosen based on EPA guidelines⁵ although smaller and larger numbers of species have been recommended (e.g., Carr and Belanger⁷). Four chemical-taxa groupings were compiled for analysis (Table 1): carbamate-all (all aquatic species); carbamate-fish (only fish

taxa); organophosphate-fish (only fish taxa); organophosphate-invertebrates (only invertebrate taxa). Due to insufficient data for an invertebrate-only carbamate group, the carbamate-all dataset was compiled instead. All analyses were conducted using the R statistical software version 4.1.0.¹²

2.3. Conventional Single-Chemical SSDs. Conventional single-chemical SSDs were generated for each compound in the four chemical-taxa groups. Following general SSD development practice as described in Posthuma et al.¹³ and Stephan et al.,⁵ we first calculated the geometric mean of toxicity values for each species within the group. Using the best-fit method described in Lambert et al.,¹ we then fitted toxicity values to four statistical distributions (log-normal, log-logistic, gamma, and Weibull) using the “fitdistrplus” package in R.¹⁴ Though other distribution-fitting methods exist, we opted to fit these symmetrical two-parameter distributions for their simplicity and to compare our results directly to those of Lambert et al.¹ We determined the best-fit distribution using the lowest Anderson–Darling statistic and extracted the HCS value from the fifth percentile of the best-fit distribution. We then extracted the upper and lower confidence limits of the best-fit distribution at the fifth percentile. We used these conventionally estimated single-chemical HCSs and confidence regions as reference values in comparing LOO- and SSDn-estimated HCSs (Table 1; Supporting Information Tables S1, S3–S6). Additionally, a preliminary censoring analysis was performed on the organophosphate-invertebrate data for dichlorvos because of a large confidence region around the HCS using the conventional fitting approach (SI-Censoring).

2.4. Single-Chemical LOO SSDs. To obtain a measure of variance around the single-chemical HCS values for each of the four chemical-taxa groups, we used LOO (N-1) mean and variance estimation. For each chemical, we recorded a list of all species with toxicity data. We removed one species from the list and created a single-chemical SSD using the best-fit method (see Methods Section 2.2). We repeated this method such that every species in the list was removed once. For example, since there were data for eight species for the chemical aminocarb, we created a total of eight SSDs such that each SSD was made up of data from seven species. For each SSD, we extracted the HCS value from the fifth percentile of the distribution. We computed the arithmetic mean and standard error of all HCSs that were extracted to get an average LOO HCS value. Variance for each average LOO HCS was computed as a 95% confidence interval around the arithmetic mean HCS (see Supporting Information Tables S3–S6).

2.5. Toxicity-Normalized Species Sensitivity Distribution. **2.4.1. Chemical Grouping and nSpecies Determination.** For each chemical-taxa group, we created all possible unique combinations of N chemicals ranging from a minimum of two compounds to a maximum of N compounds. The total number

of unique chemical combinations for a dataset with N chemicals is given as follows

total unique combinations

$$\begin{aligned} &= {}^N C_2 + {}^N C_3 + {}^N C_4 + \dots + {}^N C_N \\ &= (2^N - 1 - {}^N C_1) \end{aligned}$$

For example, the chemical-taxa group of carbamate-all contained five compounds, for which we could make 26 unique chemical combinations (10 combinations of 2 chemicals, 10 combinations of 3 chemicals, 5 combinations of 4 chemicals, and 1 combination of all 5 carbamate chemicals). For each unique combination, we determined which species had toxicity data for every chemical in that combination. We listed these species as “nSpecies candidates”. These were species that could be used as normalizing species to create an SSDn for that chemical combination, explained in the next step.

2.5.2. SSDn Data Preparation and Generation. For each unique combination (e.g., 2, 3, 4, or 5 chemicals), we picked one nSpecies (e.g., *D. magna*) from the pool of nSpecies candidates and developed an SSDn using the following steps (see Figure 1, SI-Manual, and Lambert et al.¹ for additional details). (1) We calculated the geometric mean of toxicity values for every species and chemical and set these as chemical-specific species mean acute values (SMAVs). (2) We then divided each SMAV by the SMAV of the nSpecies, resulting in toxicity-normalized SMAVs (nSMAV). For example, the nSMAV for the nSpecies will always equal 1, and the nSMAV for a species 4 times less sensitive than the nSpecies would be 4. (3) We geometrically averaged the nSMAV for each species across all chemicals within the combination to generate a single nSMAV to represent that species in the SSDn. (4) Using the same procedure as single-chemical SSD generation, we fitted the nSMAVs to four different distributions (log-normal, log-logistic, gamma, and Weibull) using the “fitdistrplus” package in R.¹⁴ We chose the best-fit distribution using the lowest Anderson–Darling statistic. (5) We extracted the toxicity-normalized fifth percentile hazard concentration (HC5n) from the fifth percentile of the best-fit distribution. (6) We repeated this process so that every possible combination of chemicals and nSpecies was used to create a separate SSDn, resulting in multiple SSDns for each chemical-taxa group.

2.5.3. HC5n and Variance Calculation. Chemical-specific HC5 values and 95 percent confidence intervals were computed from the SSDns for each chemical-taxa group as follows. (1) We extracted HC5n values from every SSDn created and then back-calculated chemical-specific HC5 values. To do this, we multiplied an HC5n value by the nSpecies SMAV for that chemical to generate a chemical-specific HC5 value, given by the following equation¹

$$HC5 = HC5n \times n\text{Species SMAV}$$

(2) From this, we obtained multiple back-calculated HC5 values for each chemical within the chemical-taxa group. For example, aminocarb in the carbamate-fish chemical-taxa group had 78 HC5 values computed across the 10 nSpecies (Figure S7). (3) For each chemical and nSpecies, we then arithmetically averaged all back-calculated HC5 values to obtain a single mean nSpecies-specific back-calculated HC5. In our example, aminocarb had 10 back-calculated HC5 values (one for each nSpecies) ranging from 500 to 8000 µg/L. (4) The arithmetic average of the chemical-specific HC5s was then computed as the final mean

back-calculated HC5 for that chemical. For example, the aminocarb final HC5 was 2360 µg/L (average of 10 HC5s). (5) We then computed the 95% confidence interval around this overall chemical-specific HC5. (6) This process was repeated for every chemical within each chemical-taxa group. For a visual example of the method, please see the user manual in the Supporting Information (SI-Manual).

2.6. Determination of HC5 Accuracy and Uncertainty.

We determined accuracy as the fold increase or fold decrease of each LOO- or SSDn-estimated HC5 relative to the single-chemical HC5. In the accuracy metric, a value of 1 represents the lowest possible value and the most accurate prediction (e.g., SSDn HC5 = SSD HC5). The uncertainty metric was computed as the ratio of the upper confidence interval to the HC5 value for SSD, LOO, and SSDn estimates (uncertainty = upper CI/HC5), which gives a simple measure of positive fold change relative to the HC5 value.

3. RESULTS

3.1. Dataset Attributes and Chemical Grouping. Of the 84 ACHEIs compiled from the Web-ICE database, 19 chemicals (5 carbamates and 14 organophosphates) had eight or more species (Table 1; Supporting Information Table S1; SI-Data). The four chemical-taxa groups assessed included the carbamate-all species (5 compounds), carbamate-fish (5 compounds) organophosphate-invertebrate (10 compounds), and organophosphate-fish (13 compounds) (Table 1). The two carbamate-taxa groups had relatively narrow ranges in toxicity values (1.7–31,000 µg/L), whereas the organophosphates had a 6 order magnitude range in fish (0.4–100,000 µg/L) and a 7 order magnitude range in invertebrate (0.04–301,000 µg/L) taxa groupings (Table 1; SI-Data).

3.2. Conventional Single-Chemical SSDs. We developed a total of 33 conventional single-chemical SSDs along with confidence regions and HC5 values (Supporting Information Tables S3–S6). The single-chemical SSD confidence regions showed generally wider ranges and higher uncertainty than the corresponding LOO and SSDn confidence intervals (Figures 2–5). The dichlorvos-invertebrate SSD had particularly high variance and an extremely low HC5 value (<0.00065 µg/L) regardless of the distribution fitting method (see Supporting Information Figure S1). Left censoring the upper quartiles of the dichlorvos-invertebrate data resulted in increasing HC5 estimates ranging from 0.010 to 0.066 µg/L (SI-Censoring).

3.3. Single-Chemical LOO SSDs. All LOO ($N-1$) HC5 estimates were highly accurate, with 100% falling within threefold of the conventionally estimated single-chemical SSD HC5 (Figures 2–5, Supporting Information Tables S2–S6). LOO HC5 estimates had very low uncertainty with 95% CI within twofold of the mean.

3.4. SSDn HC5 Values. SSDn-estimated HC5 values had high accuracy and low uncertainty, with the dichlorvos-invertebrate HC5 being the one notable exception. Of the 33 SSDn-estimated HC5 values, 76% were within threefold, 91% within fivefold, and 97% within ninefold of the conventionally estimated HC5. All 33 SSDn-estimated HC5s, including dichlorvos, had low uncertainty with 95% CI within threefold of the mean. The HC5 estimated from the dichlorvos-invertebrate SSDn was substantially higher than the conventionally estimated HC5 and was consistent with other HC5 values in the organophosphate-invertebrate group.

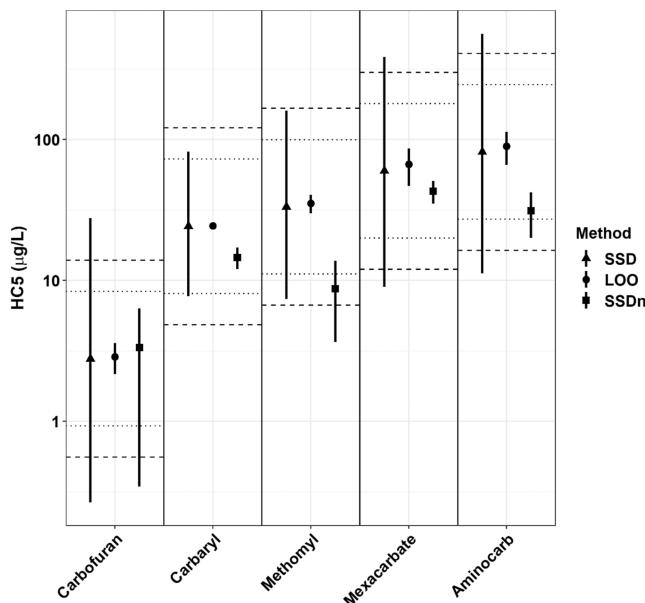


Figure 2. HC5 values for the carbamate-all species group estimated using SSD (triangle), LOO (circle), and SSDn (square) approaches. The SSD point represents a single HC5 value extracted from the best-fit distribution. The LOO point represents a mean HC5 value derived from averaging the HC5s from each LOO iteration. The SSDn point represents a mean HC5 value derived from averaging the HC5s from all nSpecies for that chemical. The dotted black lines represent threefold higher or lower than the SSD HC5, and the dashed black lines represent fivefold higher or lower than the SSD HC5. Error bars represent the 95% confidence interval around each estimate. Note that confidence intervals for each of the approaches depicted are calculated differently, see text for details.

4. DISCUSSION

A continuing challenge in ecological risk assessment is the protection of species and aquatic communities from the multitude of legacy and emerging contaminants. The SSD approach has been used internationally for over 30 years in chemical hazard estimation. The approach uses the probability distribution of toxicity values that is assumed representative of the sensitivity of a group of species to a chemical to estimate an HC5 or another percentile in the SSD. Application of SSDs in ecological risk assessment has been limited by insufficient taxonomic diversity of species to estimate representative and statistically robust HC5 values. For example, SSD-based guidance for U.S. ambient water quality criteria for aquatic life was published in 1985,⁵ but only 60 criteria values have been established during the ensuing 4 decades because of challenges in meeting minimum data requirements for taxa diversity in toxicity values. New approach methods offer potential computational solutions to filling toxicity data gaps by leveraging existing data to predict the sensitivity to new species or untested chemicals (e.g., Awkerman et al.¹⁵).

The SSDn approach is a grouped chemical method that creates a single combined SSD by normalizing toxicity data using species tested in all members of the chemical-taxa grouping. Chemical-specific HC5s are then back-calculated from the SSDn-estimated HC5 and the chemical-specific toxicity value of the normalizing species. In the current study, the SSDn approach of Giddings et al.⁸ and Lambert et al.¹ was modified to include all possible normalizing species to allow for more accurate HC5 estimation with quantifiable low uncertainty. This

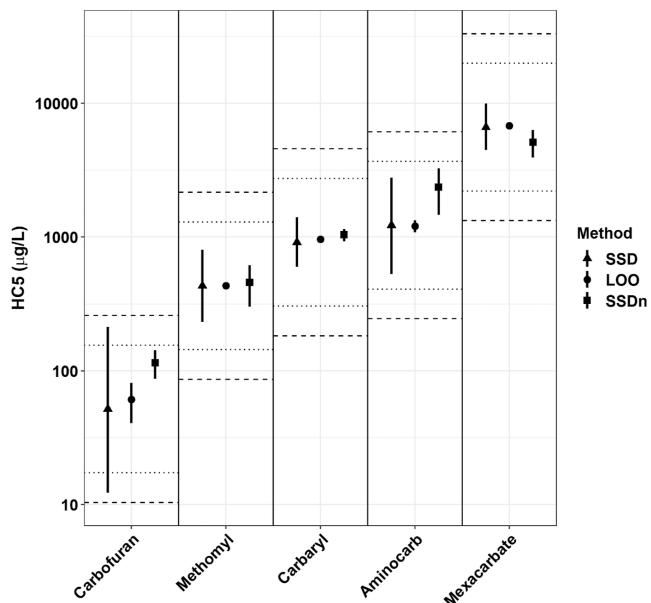


Figure 3. HC5 values for the carbamate-fish group estimated using SSD (triangle), LOO (circle), and SSDn (square) approaches. The SSD point represents a single HC5 value extracted from the best-fit distribution. The LOO point represents a mean HC5 value derived from averaging the HC5s from each LOO iteration. The SSDn point represents a mean HC5 value derived from averaging the HC5s from all nSpecies for that chemical. The dotted black lines represent threefold higher or lower than the SSD HC5, and the dashed black lines represent fivefold higher or lower than the SSD HC5. Error bars represent the 95% confidence interval around each estimate. Note that confidence intervals for each of the approaches depicted are calculated differently, see text for details.

improved SSDn approach was assessed using a case study of acute toxicity values for organophosphate and carbamate insecticides with high diversity in species composition and order of magnitude ranges in toxicity values. High accuracy and low uncertainty were demonstrated in each of the four chemical-taxa groups. Minimum acute toxicity datasets of eight species were used in each chemical-taxa group based on EPA guidelines⁵ to reduce confounding effects of inadequate data for SSD generation. Additional research is needed to assess the accuracy and uncertainty in SSDn-estimated HC5s with datasets and chemical groups with less taxa richness, fewer species, and more narrow ranges in toxicity values.

By utilizing all possible combinations of chemicals and nSpecies, the SSDn approach eliminates the requirement of a priori nSpecies assignment (as done in Giddings et al.⁸ and Lambert et al.¹) and maximizes the utility of the available data. Previous iterations of the approach derived only a single HC5 value from an SSDn with little knowledge of how it compared to the SSD or from using a different combination of chemicals. As Supporting Information Figures S2–S34 show, there is significant variation even within nSpecies HC5 estimates that is not accounted for by the previous SSDn approach that generated only a single SSDn. By leveraging multiple chemical groupings and averaging across them, a central tendency HC5 value is computed from the multiple SSDn.

Of the 33 SSDn-estimated HC5 values, only the HC5 for dichlorvos in the organophosphate-invertebrate group exhibited a large deviation from the conventional HC5 estimate. We ascribed this deviation to the highly uncertain and poorly fit conventional SSD rather than computational inaccuracy in the

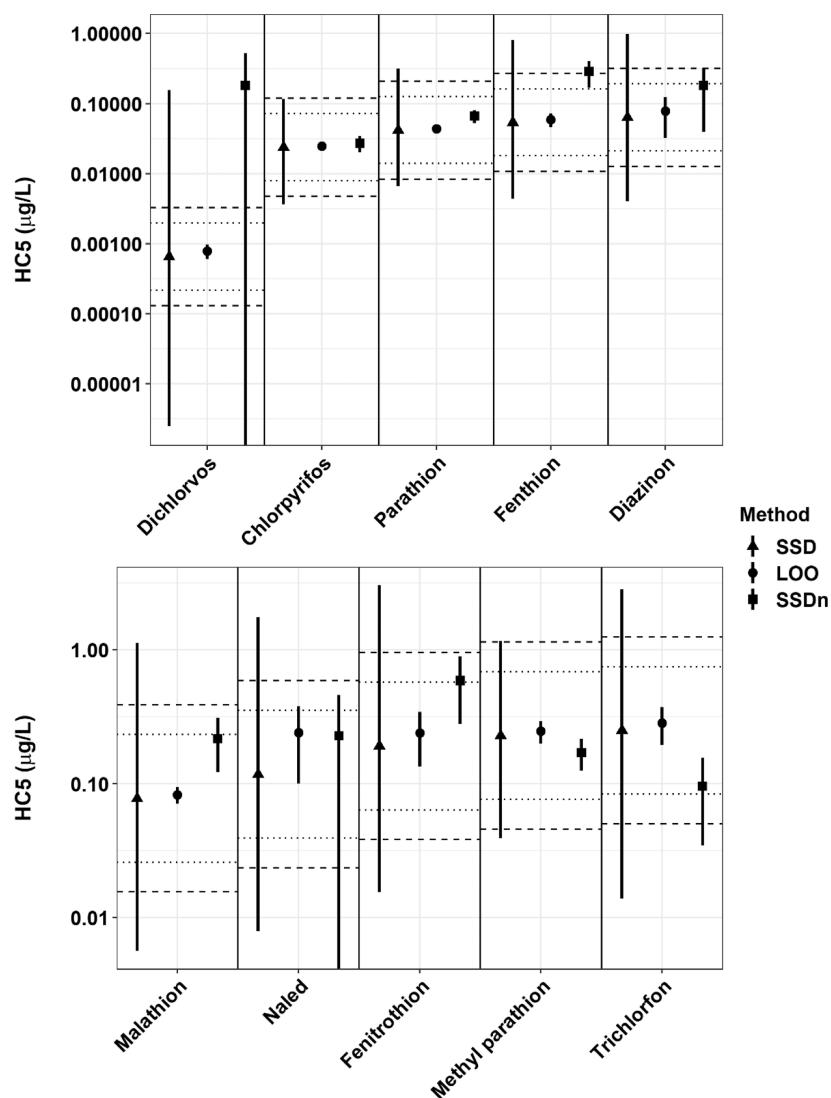


Figure 4. HC5 values for the organophosphate-invertebrate group estimated using SSD (triangle), LOO (circle), and SSDn (square) approaches. The SSD point represents a single HCS value extracted from the best-fit distribution. The LOO point represents a mean HCS value derived from averaging the HC5s from each LOO iteration. The SSDn point represents a mean HCS value derived from averaging the HC5s from all nSpecies for that chemical. The dotted black lines represent threefold higher or lower than the SSD HC5, and the dashed black lines represent fivefold higher or lower than the SSD HC5. Error bars represent the 95% confidence interval around each estimate. Note that confidence intervals for each of the approaches depicted are calculated differently, see text for details.

SSDn method. As shown in Supporting Information Figure S1, all the species in the lowest quartile of the dichlorvos conventional SSD had a within 1 order of magnitude toxicity range ($0.07\text{--}0.5\ \mu\text{g/L}$) and regardless of the distribution method, the HC5 could not be estimated with high confidence. This resulted in a conventional HC5 that was 2 orders of magnitude lower ($0.00065\ \mu\text{g/L}$) than the toxicity value for the most sensitive species ($0.07\ \mu\text{g/L}$). In contrast, the SSDn-estimated HC5 values were consistent with the SSD, LOO, and SSDn-derived values for the nine other compounds in the organophosphate-invertebrate group and exhibited low uncertainty. This illustrates a computational advantage of the SSDn approach over conventional single chemical HCS estimation achieved from leveraging data across a chemically and toxicologically similar group of compounds. Differences in nSpecies sensitivity within the chemical-taxa group accounts for the chemical-specific differences in sensitivity and the resulting compound specific HC5 estimate. Left censoring the upper

quartiles of the dichlorvos SSD data, which weights the most sensitive species more heavily, also provided an HC5 estimate more consistent with the dichlorvos SSDn estimate and the organophosphate-invertebrate group HC5 values (see Supporting Information SI-Censoring). Censoring has been only infrequently used in SSD literature because of its computational complexity but may provide an additional approach to estimating higher confidence HC5 values with highly skewed data.^{4,16}

There are several aspects of the SSDn approach that may benefit from further research. First, a major assumption of the SSDn method is similar relative sensitivity of the nSpecies across all compounds within the chemical group. However, relative nSpecies sensitivity may vary within a chemical group because of compound- or organismal-specific differences in phenotype, chemical potency, MOA, and taxa composition within each SSDn.⁸ Second, additional research is needed to better understand the minimum numbers of species and chemicals

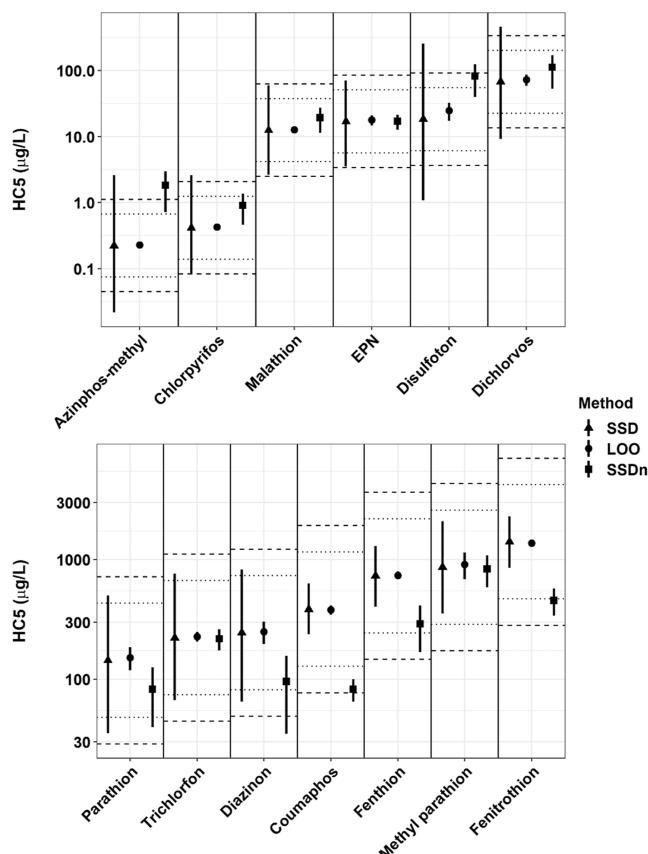


Figure 5. HCS values for the organophosphate-fish group estimated using SSD (triangle), LOO (circle), and SSDn (square) approaches. The SSD point represents a single HCS value extracted from the best-fit distribution. The LOO point represents a mean HCS value derived from averaging the HCSs from each LOO iteration. The SSDn point represents a mean HCS value derived from averaging the HCSs from all nSpecies for that chemical. The dotted black lines represent threefold higher or lower than the SSD HCS, and the dashed black lines represent fivefold higher or lower than the SSD HCS. Error bars represent the 95% confidence interval around each estimate. Note that confidence intervals for each of the approaches depicted are calculated differently, see text for details.

needed for robust SSDn estimation of an HCS. Analyses of HCS values derived from combinations of species-rich and species-poor chemicals, as well as comparison of HCS values derived from a single nSpecies, are essential next steps for applying the SSDn approach to real-world datasets that often lack adequate species diversity for HCS derivation. Third, weighting options for averaging SSDn-derived HCS values could be assessed. Larger species diversity and overall data are more likely with larger groupings of chemicals (e.g., an SSDn derived from nine chemicals will likely have more data than an SSDn derived from only two chemicals). Approaches that weight the HCSs derived from larger data combinations may provide more representative HCS estimates. In the current study, we computed the arithmetic mean of all HCS values to evenly weight each HCS when calculating variance. All the averaged HCS values were within 1–2 orders of magnitude and were nearly identical to computed geometric values. However, if HCS data had ranges of 4–5 orders of magnitude, taking the geometric mean instead may have statistical advantages. Finally, computational advances could benefit the SSDn approach. Currently, each additional chemical and nSpecies in a dataset adds exponentially more

combinations; thus, increasingly larger dataset complexity will exponentially increase computational times. Similarly, quantifying the advantages of the SSDn approach over alternative methods could be explored using simulated data with hundreds of chemicals and through Monte Carlo approaches that require higher computational power.

Traditionally, uncertainty in conventional HCS estimates has not been reported or only the confidence region of the SSD is shown. Various statistical approaches to estimating HCS uncertainty are available but have not been generally adopted in part because of their computational complexity.^{2,17–19} We developed a simple method of computing a confidence interval around the HCS using an adaptation of LOO (N-1) error estimation.^{20,21} Across all four chemical-taxa groups, LOO estimates were highly accurate and confidence intervals were extremely precise compared to the conventional SSD approach. LOO variance estimation has practical advantages over conventional SSD error estimation. In conventional SSDs, the confidence region around the HCS is based on the fitted distribution across the entire SSD rather than only the uncertainty around the HCS estimate. The resulting confidence region can be orders of magnitude larger than the HCS estimate, resulting in high uncertainty in a conventionally estimated HCS. In the current study, LOO variance estimates were substantially smaller than the corresponding confidence region of the conventional HCS, suggesting potentially higher confidence in the LOO-estimated HCS. A benefit of the LOO approach is that it allows the user to see which species are driving higher or lower HCS estimates and uncertainty when they are removed or added. This could have implications for future computational SSD approaches, where various combinations of species could be used in SSD development in deriving a better estimate of central tendency in the HCS.

There are several potential limitations of the LOO variance approach that could be addressed in the future. One limitation is the collapsed variability in LOO-derived confidence intervals, which are not determined from the fit of the distribution in the conventional HCS approach but rather from the range of HCS values generated from each LOO iteration. As such, caution should be exercised in directly comparing the confidence intervals and uncertainty around the LOO approach to conventional SSD confidence intervals despite the nearly identical HCS values determined in the current study. For a more consistent comparison to conventional SSDs, future adaptation of the LOO method could include calculation of distribution-specific confidence intervals for each LOO iteration and then developing a central-tendency confidence interval derived from combining the multiple distributions. Similarly, alternative distribution-fitting methods for the LOO approach could be explored. Multiple distribution-fitting approaches exist,¹⁷ though there has been some debate about the merits of such approaches over the past 30 years of SSD development.⁴ In all three approaches used in the current study (SSD, LOO, and SSDn), we opted to fit symmetrical, two-parameter distributions for simplicity and comparison to Lambert et al.,¹ recognizing that results may not be optimal for data with high skew. Nevertheless, our emphasis in this study was to compare the SSD and LOO approaches to the SSDn approach, and we maintained consistency in distribution fitting approaches across the three approaches.

Globally, aquatic hazard and risk assessments continue to rely on SSD-based estimates of HCSs but have been limited by adequate species number and taxa diversity. The SSDn method

has the potential for providing more accurate HCS estimates than conventional single-chemical SSDs by leveraging species toxicity data across a chemical group.^{1,8} While computationally complex, the SSDn method builds upon the basic theory and practice of conventional SSDs and is recommended for general application in SSD-based hazard estimation. To facilitate user understanding and a wider adoption of the method, a user manual, R-code files, and complete example dataset are provided in the Supporting Information. The LOO variance approach presented in the current study provides a simple computational method to estimate confidence intervals around an HCS estimate that is nearly identical to the conventionally estimated HCS. The LOO approach is recommended as an additional tool for use by practitioners of SSD-based hazard assessment to facilitate understanding uncertainty and effects of species composition on HCS estimates.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.2c06857>.

Chemical and taxa groupings of ACHEIs used in computing SSDs, HCS and LC₅₀, and species ranges of all chemicals; a glossary of terms used in the manuscript; accuracy and uncertainty metrics; comparison of poor-fitting SSD/SSDns; and raw SSDn output plots (PDF)

Raw data used to run the analysis (XLSX)

Preliminary censoring analysis (PDF)

SSDn user manual (PDF)

Code used to run the analysis (ZIP)

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Lambert, F. N.; Raimondo, S.; Barron, M. G. Assessment of a New Approach Method for Grouped Chemical Hazard Estimation: The Toxicity-Normalized Species Sensitivity Distribution (SSDn). *Environ. Sci. Technol.* **2022**, *56*, 8278–8289.
- (2) Belanger, S.; Barron, M.; Craig, P.; Dyer, S.; Galay-Burgos, M.; Hamer, M.; Marshall, S.; Posthuma, P.; Raimondo, S.; Whitehouse, P. Future needs and recommendations in the development of species sensitivity distributions: Estimating toxicity thresholds for aquatic ecological communities and assessing impacts of chemical exposures. *Integr. Environ. Assess. Manage.* **2017**, *13*, 664–674.
- (3) Del Signore, A.; Hendriks, A. J.; Lenders, H. J.; Leuven, R. S.; Breure, A. M. Development and application of the SSD approach in scientific case studies for ecological risk assessment. *Environ. Toxicol. Chem.* **2016**, *35*, 2149–2161.
- (4) Fox, D. R.; Dam, R. A.; Fisher, R.; Batley, G. E.; Tillmanns, A. R.; Thorley, J.; Schwarz, C. J.; Spry, K.; McTavish, K. Recent Developments in Species Sensitivity Distribution Modeling. *Environ. Toxicol. Chem.* **2021**, *40*, 293–308.
- (5) Stephan, C. E.; Mount, D. I.; Hansen, D. J.; Gentile, J. H.; Chapman, G. A.; Brungs, W. A. *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*; US Environmental Protection Agency: Washington, DC, 1985; p 98.
- (6) European Chemicals Agency. *Guidance on Information Requirements and Chemical Safety Assessment*, 2008; Chapter R.10: Characterization of dose [exposure]-response for environment. Helsinki, Finland. [cited 2022 October 31]. Available from. <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>.
- (7) Carr, G.; Belanger, S. SSDs Revisited: Part I—A Framework for Sample Size Guidance on Species Sensitivity Distribution Analysis. *Environ. Toxicol. Chem.* **2019**, *38*, 1514–1525.
- (8) Giddings, J. M.; Wirtz, J.; Campana, D.; Dobbs, M. Derivation of combined species sensitivity distributions for acute toxicity of pyrethroids to aquatic animals. *Ecotoxicology* **2019**, *28*, 242–250.
- (9) Coleman, A. L.; Edmands, S. Data and Diversity in the Development of Acute Water Quality Criteria in the United States. *Environ. Toxicol. Chem.* **2022**, *41*, 1333–1343.
- (10) Willming, M. M.; Lilavois, C. R.; Barron, M. G.; Raimondo, S. Acute Toxicity Prediction to Threatened and Endangered Species Using Interspecies Correlation Estimation (ICE) Models. *Environ. Sci. Technol.* **2016**, *50*, 10700–10707.
- (11) Raimondo, S.; Lilavois, C. R.; Barron, M. G. *Web-Based Interspecies Correlation Estimation (Web-ICE) for Acute Toxicity: User Manual*. Version 3.3, EPA/600/R-15/192; U. S. Environmental Protection Agency, Office of Research and Development, Gulf Ecology Division: Gulf Breeze, FL, 2015.
- (12) R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2022; (<https://www.R-project.org/>)
- (13) Posthuma, L.; SuterII, G. W.; Traas, T. P., Eds. *Species Sensitivity Distributions in Ecotoxicology*, 1st ed.; CRC Press, 2001.
- (14) Delignette-Muller, M. L.; Dutang, C. fitdistrplus: An R Package for Fitting Distributions. *J. Stat. Software* **2015**, *64*, 1–34.
- (15) Awkerman, J. A.; Raimondo, S.; Jackson, C. R.; Barron, M. G. Augmenting aquatic species sensitivity distributions with interspecies toxicity estimation models. *Environ. Toxicol. Chem.* **2014**, *33*, 688–695.
- (16) Kon Kam King, G.; Veber, P.; Charles, S.; Delignette-Muller, M. L. MOSAIC_SSD: A new web tool for species sensitivity distribution to include censored data by maximum likelihood. *Environ. Toxicol. Chem.* **2014**, *33*, 2133–2139.
- (17) Schwarz, C. J.; Tillmanns, A. R. Improving statistical methods to derive species sensitivity distributions. *Water Science Series*. WSS2019-07, Province of British Columbia, Victoria. Available from, 2019;

https://a100.gov.bc.ca/pub/acat/documents/r57400/2_1568399094009_8398900200.pdf.

- (18) Ciffroy, P.; Keller, M.; Pasanisi, A. Estimating hazardous concentrations by an informative Bayesian approach. *Environ. Toxicol. Chem.* **2013**, *32*, 602–611.
- (19) Zajdlik, B. A. A statistical evaluation of the safety factor and species sensitivity distribution approaches to deriving environmental quality guidelines. *Integr. Environ. Assess. Manage.* **2016**, *12*, 380–387.
- (20) Krzanowski, W. J.; Hand, D. J. Assessing error rate estimators: the leave-one-out method reconsidered. *Aust. N. Z. J. Stat.* **1997**, *39*, 35–46.
- (21) Zollanvari, A.; Braga-Neto, U. M.; Dougherty, E. R. On the sampling distribution of leave-one-out error estimators for linear classifiers. *Pattern Recogn.* **2009**, *42*, 2705–2723.