

Consideration of Species Selection and Endpoint Design in the Construction of Species Sensitivity Distributions.

Michael J. Bradley, Melissa L. Staggs Smithers Viscient, Wareham, MA

Abstract

The risk of plant protection products (PPP) to aquatic organisms in Europe can be evaluated through a three tier process. Tier I testing evaluates risk to a small number of standard guideline species and conservative assessment factors are applied to determine the regulatory acceptable concentration (RAC). If predicted environmental concentrations exceed the RAC, the evaluation of risk must be refined, or use patterns of the PPP must be modified to reduce risk. Tier II testing provides the option of refining risk based on additional laboratory testing, while Tier III testing evaluates risk based on model ecosystem testing. Tier III provides the greatest opportunity for refinement, however, these experiments are quite labor and data intensive and interpretation may be subjective. Tier II testing largely revolves around standardized laboratory testing with potentially non-standard species to better understand taxonomic risk. If multiple species of a similar taxon can be evaluated, a species sensitivity distribution (SSD) can be used to improve the RAC. When considering non-standard species, the direct applicability of an established guideline or test method is unlikely, so what considerations must be made when evaluating the right test designs and candidates for construction of an SSD?

Typical Tier I Testing			Tier II SSD Refinement		
Taxa	Test Type	Endpoint	Assessment Factor (AF)	Minimum Species Required	Refined Assessment Factor
Vertebrate	Fish Acute	LC ₅₀	100	5	9
	Fish Early Life Stage ^a	EC ₁₀	10	5	3
Invertebrate	Arthropod Acute (Daphnid) ^b	LC ₅₀	100	8	3-6
	Arthropod Chronic (Daphnid) ^a	EC ₁₀	10	8	3
Primary Producer	Green Algae Toxicity ^c	EC ₅₀	10	8	3

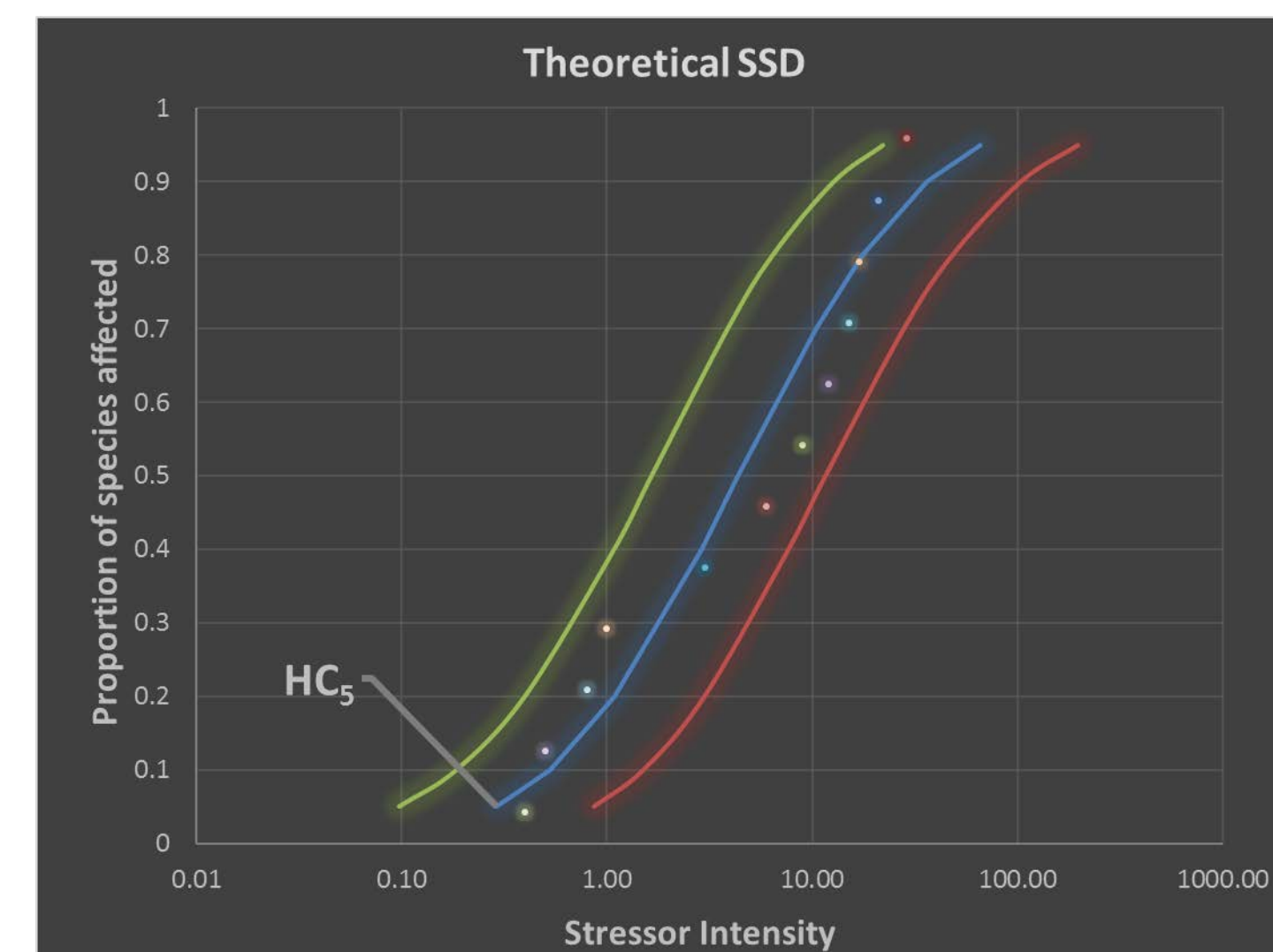
^a Hydrolysis DT₉₀ > 24 hours

^b Additional standard arthropod, (e.g., chironomid or mysid shrimp) required for insecticidal mode of action

^c Additional algae (non-green algae) and *Lemna sp.* required for herbicidal mode of action

Application of the SSD

A species sensitivity distribution is created by ranking the response for a single stressor of multiple species and plotting them on a log scale. Based on the distribution, the hazard concentration to 5% of the species tested (HC₅) is determined. The Tier II assessment factor is then applied to the HC₅ (HC₅÷AF) to determine the new RAC.



SSD Basic Guidelines

Taxonomic Relevance – Species selected should be reasonable surrogates for vulnerable, ecologically important populations, that are also, preferably, geographically relevant.

Taxonomic Specificity – Combining taxonomic classes or orders should initially be avoided, unless comparable sensitivity or general biocidal activity is established (e.g., first evaluate crustaceans and insects separately, as opposed to arthropods as a whole).

Endpoint Relevance – Endpoints used to populate an SSD must be toxicologically and ecologically relevant, and based on the most sensitive endpoint (typically identified in Tier I). Data used in the SSD must be of the same measure (e.g., growth or reproduction, not a combination of both).

Comparable Sensitivity – Data that populate the curve are ideally distributed across the entirety of the curve and not skewed to a particular end. All data points are preferably defined and bound (i.e., not < or > values), although this may not be possible in all instances.

Maintenance of Overall Protection Goals – Refined assessment factors for a specific taxon cannot result in a RAC that is less protective than Tier I RACs established for other taxa, i.e., all RAC's must remain protective of vertebrates on the individual basis, and plants and invertebrates on a population basis.

Test Design Objectives

The ultimate goal of the test design should be to establish well defined endpoints that can be objectively evaluated to meet regulatory rigor. To do this, the following aspects should be considered during development of the test design:

Testing Guidance	Acceptability	Organism Availability	Organism Performance
Is there a guideline reference for comparison and evaluation?	Are there defined performance criteria?	Is the species readily available and verifiable?	Is there a historical base-line for performance?
Is there an aspect of the guidance that must be modified?	Are there exceptions that must be considered?	Is the source reputable or reliable?	Are all defined endpoints robust and achievable?

Test Design Resources

Guidance Type	Examples	Advantages	Disadvantages
Formal Guidelines	OECD Guidelines	<ul style="list-style-type: none"> Prescriptive test design Defined endpoints Defined criteria Validated Designed to meet regulatory rigor 	<ul style="list-style-type: none"> May not be readily applicable to alternate species Adaptations/deviations to accommodate alternate species may be scrutinized
	OCSPP Guidelines	<ul style="list-style-type: none"> Prescriptive test design Defined endpoints Some defined criteria Often provide background development and design for multiple species Provide “proof of concept” for some non-standard species 	<ul style="list-style-type: none"> May be incomplete Not necessarily validated Some information may be anecdotal and not transferable Information may be outdated May not meet regulatory rigor
Test Methods	ASTM	<ul style="list-style-type: none"> Prescriptive test design Defined endpoints Some defined criteria Often provide background development and design for multiple species Provide “proof of concept” for some non-standard species 	<ul style="list-style-type: none"> May be incomplete Not necessarily validated Some information may be anecdotal and not transferable Information may be outdated May not meet regulatory rigor
	EPA WET Methods	<ul style="list-style-type: none"> Large library of non-standard species and test designs Provides baseline for applicability and feasibility 	<ul style="list-style-type: none"> Materials and Methods may lack detail Uncertainty of repeatability May lack defined endpoint criteria
Open Literature	Presentations & Publications	<ul style="list-style-type: none"> Large library of non-standard species and test designs Provides baseline for applicability and feasibility 	<ul style="list-style-type: none"> Materials and Methods may lack detail Uncertainty of repeatability May lack defined endpoint criteria
Consultation	Commercial Suppliers	<ul style="list-style-type: none"> Provides intimate knowledge of necessary conditions & capability Provides some expectation of performance 	<ul style="list-style-type: none"> Information may be largely culture based and not directly applicable to testing (different objectives)
	Hobbyists	<ul style="list-style-type: none"> Provides intimate knowledge of necessary conditions & capability Provides some expectation of performance 	<ul style="list-style-type: none"> Information may be largely culture based and not directly applicable to testing (different objectives)

Endpoint Comparisons

Survival	Growth	Reproduction
<ul style="list-style-type: none"> Survival Immobilization Hatch Emergence 	<ul style="list-style-type: none"> Length Weight Biomass Growth Rate Time to development (emergence, first brood) Yield 	<ul style="list-style-type: none"> Total young Total eggs Young per adult/female Eggs per adult/female Percent hatch Mating success Fertilization

- The endpoint used to construct an SSD should be the most sensitive category (survival, growth, or reproduction).
- Test designs should capture common endpoints with similar units of measure among all species where possible.
- The selected endpoints should be suitable to define a robust dose response (preferably support EC_x calculations).

Species Selection

Taxa	Common Division	Examples	Advantages	Limitations
Vertebrates	Fish	<ul style="list-style-type: none"> <i>P. promelas</i> (Fathead) <i>O. mykiss</i> (Rainbow Trout) <i>C. variegatus</i> (Sheepshead) <i>O. latipes</i> (Medaka) <i>D. rerio</i> (Zebra fish) 	<ul style="list-style-type: none"> Acute and chronic guidance are available and applicable to a number of species Acute data are commonly produced and available 	<ul style="list-style-type: none"> EU legislation prohibits use of invertebrates beyond what is required by regulation This limits opportunity to construct an SSD with new data
Arthropod Invertebrates	Aquatic Insects	<ul style="list-style-type: none"> Chironomids (midge) <ul style="list-style-type: none"> <i>C. dilutus</i>, <i>C. riparius</i> Ephemeroptera (mayflies) Plecoptera (stoneflies) Trichoptera (caddisflies) (EPT) 	<ul style="list-style-type: none"> Chironomids: <ul style="list-style-type: none"> Readily cultured Variety of validated methods for survival, growth, and reproduction EPT: <ul style="list-style-type: none"> Highlighted as vulnerable and sensitive to contaminant stressors Strengthen assessment & can improve assessment factors 	<ul style="list-style-type: none"> EPT: <ul style="list-style-type: none"> Often seasonally limited Nymph stages can be multiple years which limits life-cycle assessment testing Limited experience/data on stoneflies Long term test methods for mayflies exist but are highly specialized
	Crustacea	<ul style="list-style-type: none"> Cladocerans (Daphnids, Fairy shrimp) Mysids Amphipods (Gammarids) Rotifers Isopods (water louse) Copepods Decapods (true shrimp, prawns) 	<ul style="list-style-type: none"> Validated formal guidelines for many (Daphnia, mysids, amphipods) Applicable test methods for others (rotifers, copepods, decapods) Organisms of all classes are readily cultured in mass Easily acquired for acute testing 	<ul style="list-style-type: none"> Chronic Testing: <ul style="list-style-type: none"> Comparable endpoints are not available for all (feasibility of growth or undefined reproduction time of non-standard species) Exposure designs are often specialized for each species and labor intensive
Other Invertebrates	Annelids	<ul style="list-style-type: none"> Oligochaetes <ul style="list-style-type: none"> <i>L. variegatus</i>, <i>T. tubifex</i> Polychaetes 	<ul style="list-style-type: none"> Easily cultured Applicable guidelines and test methods (acute and chronic) Simple exposure designs 	<ul style="list-style-type: none"> Historically less sensitive than other invertebrates Potential to skew or not fit distribution
	Mollusca	<ul style="list-style-type: none"> Bivalves (oysters, mussels) Gastropods (snails) 	<ul style="list-style-type: none"> Standard acute and chronic guidance available 	<ul style="list-style-type: none"> Some guideline species are restricted (invasive; <i>L. stagnalis</i>, <i>P. antipadourum</i>) Limited reproduction endpoint
Primary Producers	Algae	<ul style="list-style-type: none"> Green Algae <ul style="list-style-type: none"> <i>R. subcapitata</i>, <i>D. subspicatus</i> Cyanobacteria <ul style="list-style-type: none"> <i>A. flos-aque</i>, <i>S. leopoliensis</i> Diatoms <ul style="list-style-type: none"> <i>N. pellicosa</i>, <i>S. costatum</i> 	<ul style="list-style-type: none"> Standardized guidelines Many species with long history of use under protocol Many species are readily available with standardized culture practices 	<ul style="list-style-type: none"> Some species may not perform to guideline specifications/criteria This should be addressed preemptively when anticipated
	Aquatic Plants	<ul style="list-style-type: none"> Monocots <ul style="list-style-type: none"> <i>Lemna sp.</i>, <i>G. maxima</i>, <i>E. canadensis</i> Dicots <ul style="list-style-type: none"> <i>Myriophyllum sp.</i>, <i>C. demersum</i> 	<ul style="list-style-type: none"> Standardized guidance available for some species Dependent on mode of action (MOA), may be combined with algae to populate SSD (opportunity for efficiency) 	<ul style="list-style-type: none"> Specific MOA may require evaluating monocots or dicots specifically, reducing pool of candidates Species limitations due to invasiveness Sensitivity can vary by season and/or geographical source

Conclusion

The effectiveness and acceptability of an SSD relies on the suitability of the individual studies that it is comprised of. Test designs must be criteria driven with clearly defined endpoints to enable objective evaluations. Organisms selected must be reliable and reasonably sensitive.

The strength of an SSD will often increase with the number of organisms added, however, it is necessary to understand test design and organism limitations ahead of an effort to define numerous measures of taxonomic sensitivity.