The Relative Sensitivity of Macrophyte and Algal Species to Herbicides and Fungicides: An Analysis Using Species Sensitivity Distributions

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ABSTRACT

In January 2008, at a SETAC workshop on Aquatic Macrophyte Risk Assessment for Pesticides (AMRAP), a Species Sensitivity Distribution (SSD) working group was formed to address uncertainties about the sensitivity of *Lemna* and other standard test species to pesticides relative to other aquatic macrophyte species. For 11 herbicides and 3 fungicides for which relevant and reliable data were found for at least 6 macrophyte species (considered the minimum for SSD analysis), SSDs were fitted using lognormal regression. The position of *L. gibba* in each SSD was determined. The sensitivity of standard algal test species relative to the macrophytes in each SSD was also considered (algae were not included in the SSD). In recognition of the known sensitivity of *Myriophyllum* species to some herbicides and ongoing activities to develop standardized test methods for these species, the position of *M. spicatum* in each SSD was also determined where data were available. Results indicated that *L. gibba* is among the most sensitive macrophyte species for approximately half of the chemicals examined. In the majority of cases, the lowest standard algal test species endpoint was lower than the most sensitive macrophyte endpoint. *M. spicatum* was among the most sensitive macrophytes for approximately one-quarter of the chemicals. While no single species consistently represents the most sensitive aquatic plant species, for 12 out of 14 compounds algae and *L. gibba* include an endpoint that is near or below the 5th percentile of the macrophyte SSD. For the other two compounds, *M. spicatum* is the most sensitive species of all aquatic plants considered.

BACKGROUND AND OBJECTIVE

For various practical and historical reasons, the macrophytes most widely used in toxicity tests with pesticides are duckweeds of the genus *Lemna*. However, the sensitivity of *Lemna* spp. relative to other macrophyte species is largely unknown. The primary objective of the SSD working group was to investigate this question using available data on the toxicity of pesticides, especially herbicides, to aquatic macrophytes.

METHODS

We collected macrophyte and algal toxicity data for nearly 60 herbicides and fungicides from the open literature and confidential company reports. We reviewed each data source according to predefined criteria, and only data from studies determined to meet the usability criteria were included in the analysis. (In a few cases data were taken from reliable secondary sources and data quality was not independently confirmed.) For 11 herbicides and 3 fungicides, usable toxicity data were found for at least 6 macrophyte species, which was considered the minimum needed for SSD analysis.

Macrophyte SSDs for 13 of these chemicals were fitted using lognormal regression functions in EXCEL®. (For one chemical, too many "greater-than" values prevented calculation of an SSD.) The position of *Lemna gibba* in each SSD, as well as the sensitivity of the 4 algal test species required for pesticide registration in the United States under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) relative to the macrophytes in each SSD, were determined. The position of a rooted macrophyte species, *Myriophyllum spicatum*, was also determined where data were available, because a standardized *Myriophyllum* test is currently under development through another AMRAP working group (Maltby et al. 2010; Dohmen 2010) and through a UBA ring-test (Maleztki and Kussatz 2011; Maleztki et al. 2011) and is recommended under the recent SANCO 11802-2010 draft regulation.

To maintain the confidentiality of data provided by pesticide registrants, the chemicals are identified by codes that indicate the mode of action (MoA) of each chemical but not its specific identity. The MoAs included inhibition of amino acid synthesis (Chemical A), auxin simulation (Chemical B), inhibition of cell division or elongation (Chemical C), inhibition of fungal respiration (Chemicals D1 and D2), inhibition of multiple biosynthesis pathways (Chemicals E1, E2, E3, and E4), and inhibition of photosynthesis (Chemicals F1, F2, F3, F4, and F5). Because the six MoAs were not equally represented in the database and three were represented by only a single chemical, conclusions about the relationship between MoA and species sensitivity must be made with caution.

DATA SELECTION

The database contained a variety of statistical endpoints, but only median effect concentrations (EC50s) were available for a sufficient number of species to support SSD analysis. The EC50s were based on a wide variety of biological measurements, and these had to be pooled for SSD analysis. Basing SSDs on a variety of measurements was necessary for two reasons. First, differences in biology of the test species necessitate differences in measured responses (e.g. frond number, root length, plant dry weight); to construct an SSD that includes macrophytes with different morphology and growth characteristics *requires* the use of differently-derived EC50s for different species. Second, as a practical matter, subdividing the database by categories of measured data points severely reduces the number of SSDs that can be evaluated, because equivalent data are often unavailable for 6 or more species.

Given the difficulties of restricting data selection for SSDs based on categories of measurement data points, the **SSDs examined in this project used the lowest reported EC50 for each species**, regardless of the biological measurement upon which the EC50 was based. While selection of the lowest available EC50 is standard regulatory practice (e.g., US EPA 2004), it leaves open the possibility that a data point based on a non-standard measurement parameter could unduly influence the SSD.

SSD ANALYSIS

- Select toxicity endpoint for each species.
- Rank endpoints, lowest to highest.
- Calculate percentile *p* for each endpoint: $p = i/(N+1)$
  where *i* = endpoint rank, N = # of endpoints.
- Normalize *p* (Excel NORMSINV function).
- Model SSD by linear regression of NORMSINV(*p*) vs log(conc)

USABILITY CRITERIA

Each study was rated for usability according to the following criteria.

- Test organisms must be identified at least to genus.
- Test substance must be identified (active ingredient, form).
- Test substance must not include more than one active ingredient.
- Negative and/or solvent controls (as appropriate) must be included.
- Exposure medium must be reported.
- Exposure duration must be specified.
- Methods for measuring effects must be described.
- Test concentration units must be unambiguous.
- Toxicity endpoint (e.g., EC50, NOEC) must be reported or calculable from data presented.

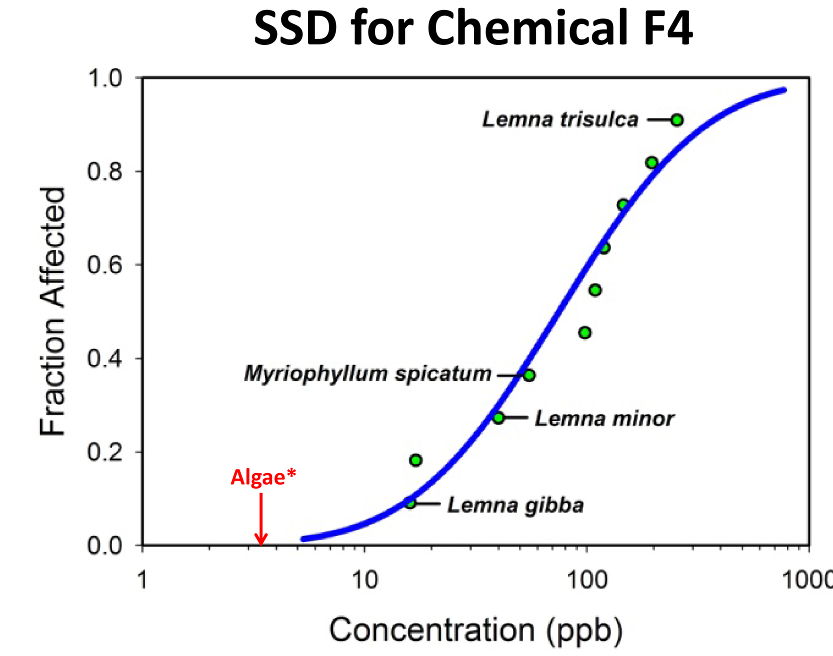
EVALUATION CRITERIA

Each usable study was evaluated according to the following criteria.

- Were the data derived using a standard, validated test method?
- Was the source of test organisms described?
- Were the plants maintained under appropriate conditions before use in the test?
- Were the test organisms healthy at the beginning of the exposure period?
- Did the study include multiple exposure concentrations?
- Were exposure concentrations confirmed by chemical analysis?
- Are response measurements reported for each exposure concentration, or only statistical endpoints such as EC50 or NOEC values?
- Are response measurements for controls and treatment groups reported?
- Is control response acceptable?
- Are methods documented sufficiently?

SSD Examples

SSD for Chemical F4



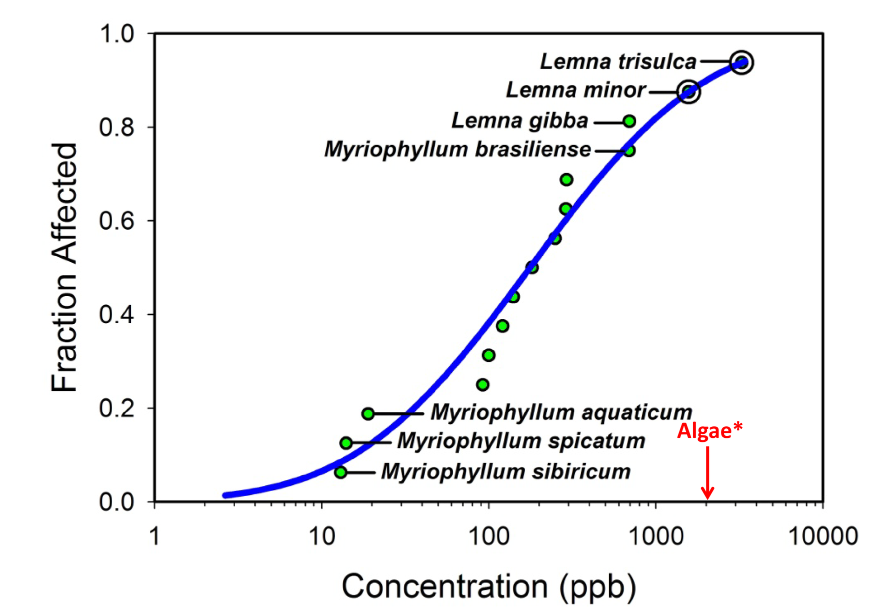
N	r^2	HC5 (ppb)	HC50 (ppb)
10	0.9366	10 (4.8-22)	75 (39-142)

	Lemna gibba	*Myriophyllum spicatum*	Most sensitive algae[1]*
Fraction Affected	9.1%	36.4%	0.43%
EC50/HC5	1.5	5.3	0.3
EC50/lowest EC50	1.0	3.4	0.2

[1]*Pseudokirchneriella subcapitata* EC50 = 3.2 ppb

* Algae were not included in the SSDs.

SSD for Chemical B



N (total)	N (regression)	r^2	HC5 (ppb)	HC50 (ppb)
15	13	0.9390	7.7 (3.0-20)	177 (78-400)

	Lemna gibba	*Myriophyllum spicatum*	Most sensitive algae[1]*
Fraction Affected	81.3%	12.5%	90.9%
EC50/HC5	90	1.8	261
EC50/lowest EC50	54	1.1	155

[1]*Navicula pelliculosa* EC50 = 2020 ppb

FINDINGS

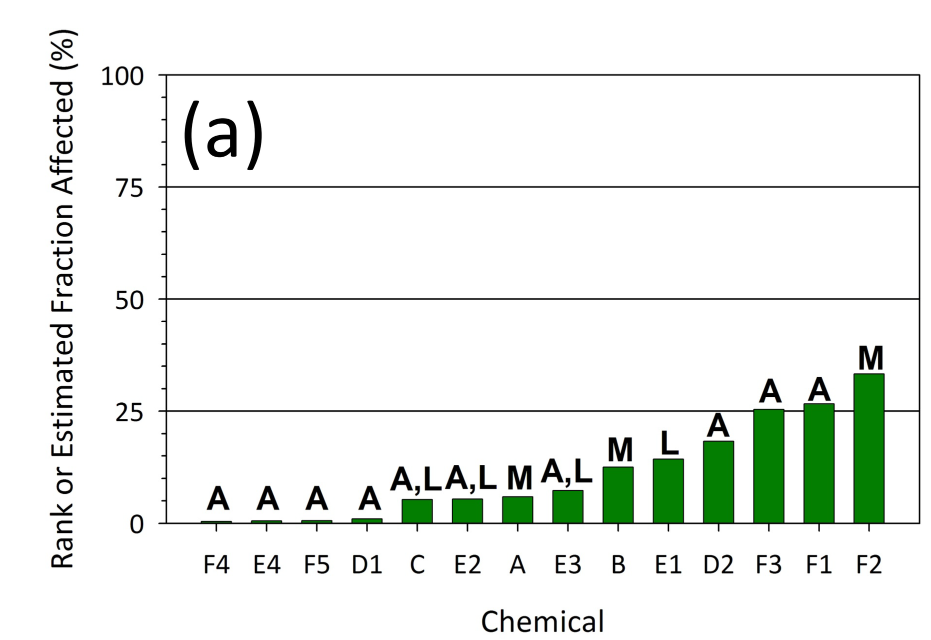
(a) When the most sensitive species of interest (i.e., *L. gibba*, the 4 FIFRA algae, and *M. spicatum*) are considered, the lowest of the EC50s is within the lower 25th percentile of the macrophyte SSD for nearly all chemicals.

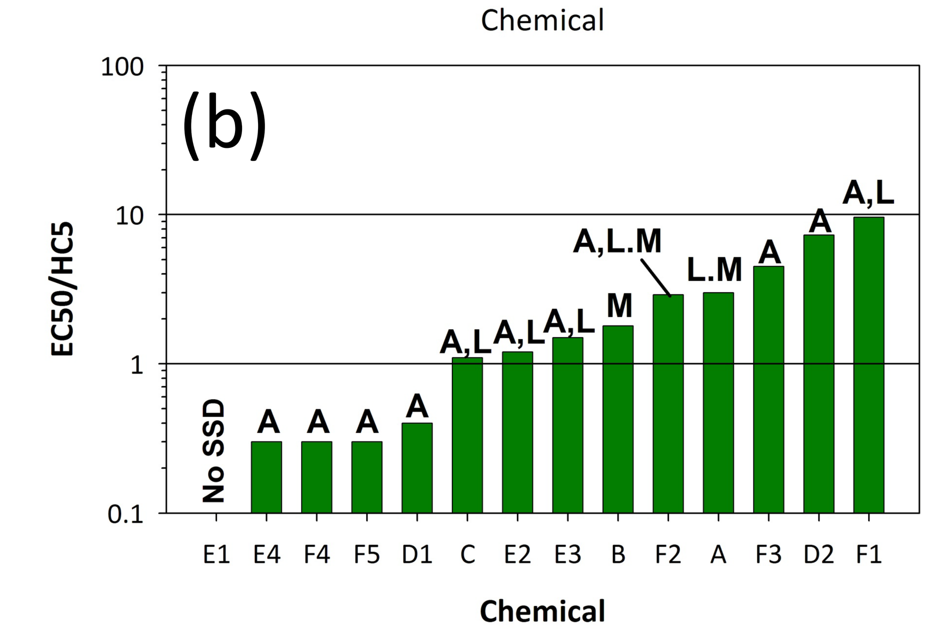
(b) The EC50 for the most sensitive of these species is at or below the corresponding macrophyte HC5 for 6 of 14 chemicals. The lowest EC50 of these species is within a factor of 10 of the HC5 for all chemicals.

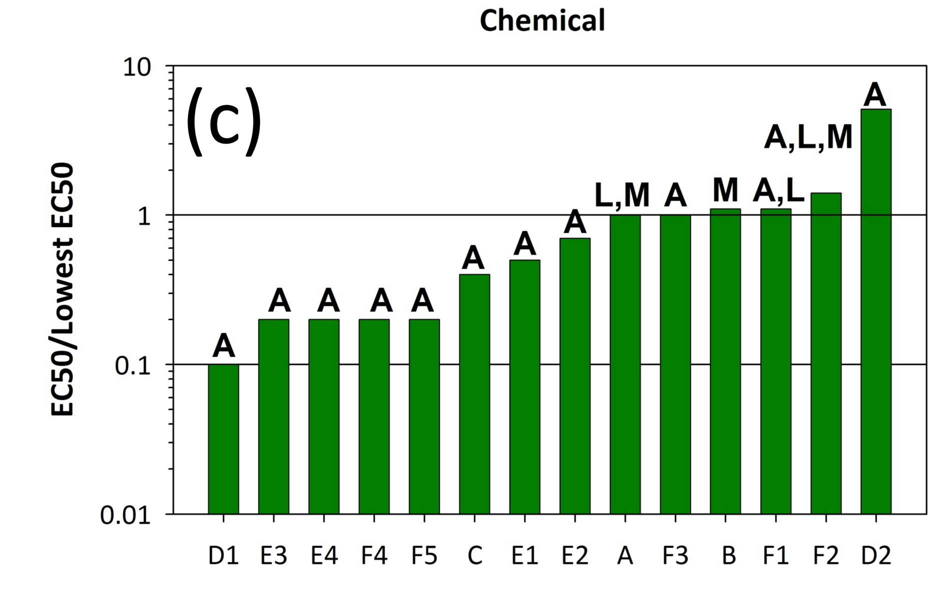
(c) The lowest of these EC50s is at or below the EC50 of the most sensitive macrophyte for all chemicals except Chemicals D2 and F2. Even for these exceptions, the difference is within a factor of 5.

CONCLUSIONS

- Neither *Lemna gibba* nor *Myriophyllum spicatum* is consistently among the most sensitive macrophyte species for all herbicides and fungicides.
- *Lemna gibba* is among the most sensitive macrophyte species for approximately half of the herbicides and fungicides examined. *L. gibba* is quite insensitive to about a quarter of the chemicals.
- *M. spicatum* is among the most sensitive macrophyte species for approximately one-quarter of the herbicides and fungicides examined. *M. spicatum* is among the least sensitive macrophytes to several others.
- For a majority of the chemicals examined, the most sensitive of the FIFRA algal species is more sensitive than the most sensitive macrophyte. In a few cases, the tested algae are much less sensitive than most macrophytes.
- **While no single species consistently represents the most sensitive macrophyte species, the combination of *L. gibba* and the 4 FIFRA algae almost always includes a data point that is near or below the most sensitive macrophyte data point and the macrophyte HC5.**
- **For the exceptional chemicals for which the EC50s of *L. gibba* and the FIFRA algae are not near or below the most sensitive macrophyte EC50, *M. spicatum* is among the most sensitive species.**
- These conclusions are subject to the limitations of the available data. This analysis is based on chemicals representing 6 different modes of action but some modes of action are represented by only one chemical. As data become available for additional chemicals, it may be possible to refine the analysis.







A, L, and M indicate that the lowest value for each chemical is based on algae (FIFRA species), *Lemna gibba*, or *Myriophyllum spicatum*, respectively. In some cases two or three of these species are similar in sensitivity, as indicated by multiple letters separated by commas.

The report, "**The Relative Sensitivity of Macrophyte and Algal Species to Herbicides and Fungicides: An Analysis Using Species Sensitivity Distributions,**" is available from the author and will also be made available through the SETAC Aquatic Macrophyte Ecotoxicology Group (AMEG).

Myriophyllum spicatum
(Nina Cedergreen, U. Copenhagen)

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