A photograph of a pond with several lily pads. In the center, a white water lily flower is in full bloom. The water is dark blue, and the lily pads are various shades of green and brown. The text is overlaid on the image.

Exploration of Methods for Characterizing Effects of Chemical Stressors to Aquatic Plants

Laura Dobbins, Mike Lewis,
Sujatha Sankula, Glen Thursby

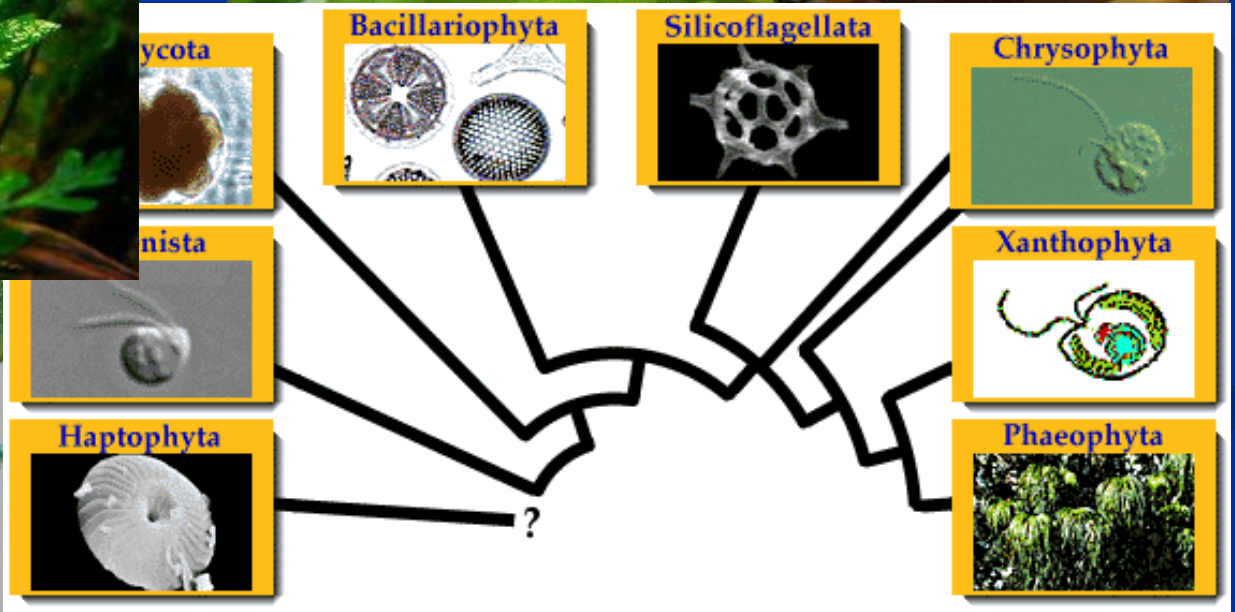
National Stakeholder Meeting
December 1, 2010

Outline

- Purpose and Scope
- Existing approaches
- Plant relative sensitivity
- Proposed approaches

Plant Community

What is a plant?



Scope of Plant Methodology

- Consider existing approaches used by OW, OPP and others for characterizing plant aquatic ecological effects
- Describe the best integrated use of existing tools for incorporating plant effects into aquatic community-level screening values
- Characterize the uncertainty and robustness of current data for aquatic plants

OPP's Approach to Evaluate Aquatic Plant Effects

- Tier I (Limit test)
 - Needed for all pesticides with outdoor uses
 - 4 microalgae + *Lemna*: laboratory tests with Technical Grade Active Ingredient (TGAI)
 - If >50% effect, Tier II testing required
- Tier II (Dose-response test)
 - Pesticides that are known phytotoxins also tested at Tier II
 - 4 microalgae + *Lemna*: laboratory tests with TGAI
- Tier III (Field test)
 - 4 vascular plant families, 3 seedless vascular plant families, 10+ families of algae, 1 bryophyte family tested with typical end-use product to determine detrimental effects at critical growth stages
 - Rarely required by the Agency

Typical Aquatic Plant Surrogates Used in US Regulatory Testing

Non-vascular plants



Pseudokirchneriella subcapitata



Anabaena flos-aquae



Navicula pelliculosa



Skeletonema costatum

Lemna gibba,
a free-floating vascular macrophyte



OW's Approach to Evaluate Aquatic Plant Effects

- Minimal plant data are required for the derivation of Water Quality Criteria
- “Results of tests with plants usually indicate that criteria which adequately protect aquatic animals and their uses will probably also protect aquatic plants and their uses.”
 - May not be supported when addressing certain chemical classes (e.g., herbicides)
- Plant value based on a 96-hr test conducted with an alga or a chronic test conducted with an aquatic vascular plant
- Final Plant Value: lowest value from a test with an “important” plant species where test concentrations are measured, and endpoint is biologically “important”.

Approaches Used Internationally

■ Canada

- At least one vascular plant or alga to derive guidelines (if the compound is highly phytotoxic, 4 species are required)
- SSDs
- Safety factors

■ European Union

- Requires a green algae test (for herbicides, tests on an alga and a vascular plant)
- SSDs
- Safety factor

State Approach (MN)

- Derivation of standards for herbicides acetochlor and metolachlor
- Protection goals:
 - Protect overall integrity of plant community, avoiding negative shifts in species composition
 - Protect most sensitive species, if it is ecologically important
 - Target 20th percentile level of protection (5th percentile of EC50 values or 20th percentile of MATC values)

State Approach (MN)

- Acute criterion derived using Great Lakes Initiative Tier II methodology with standard animal data
- Chronic criterion derived using distribution of plant data only
- Both EC_{50} values and/or maximum acceptable toxic concentration (MATCs) were collected and put in separate distributions

State Approach (MN)

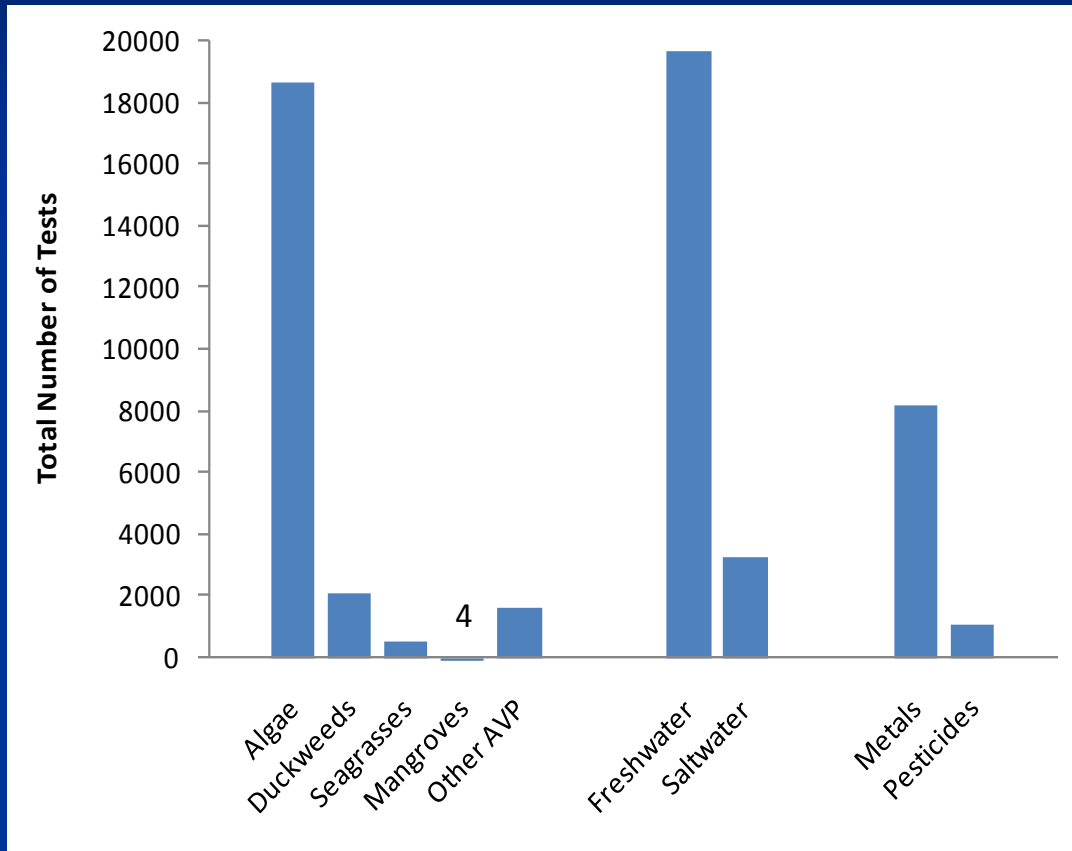
- Different endpoints and durations were combined to create SSDs
- Percentile value was chosen based on data set robustness or statistical considerations

Parameter	Acetochlor		Metolachlor	
	N	Value (ug/L)	N	Value (ug/L)
5 th %tile of EC50	8	0.093	18	35.6
20 th %tile of MATC	8	1.74	9	11.1
Final Criterion		3.6		23

Sources of Uncertainty

- Toxicity study endpoints
 - Type of effect
 - Summary statistics
 - Duration
- Relative Sensitivity of standard test species
 - Physiology
 - Habitat

Standard Plant Data

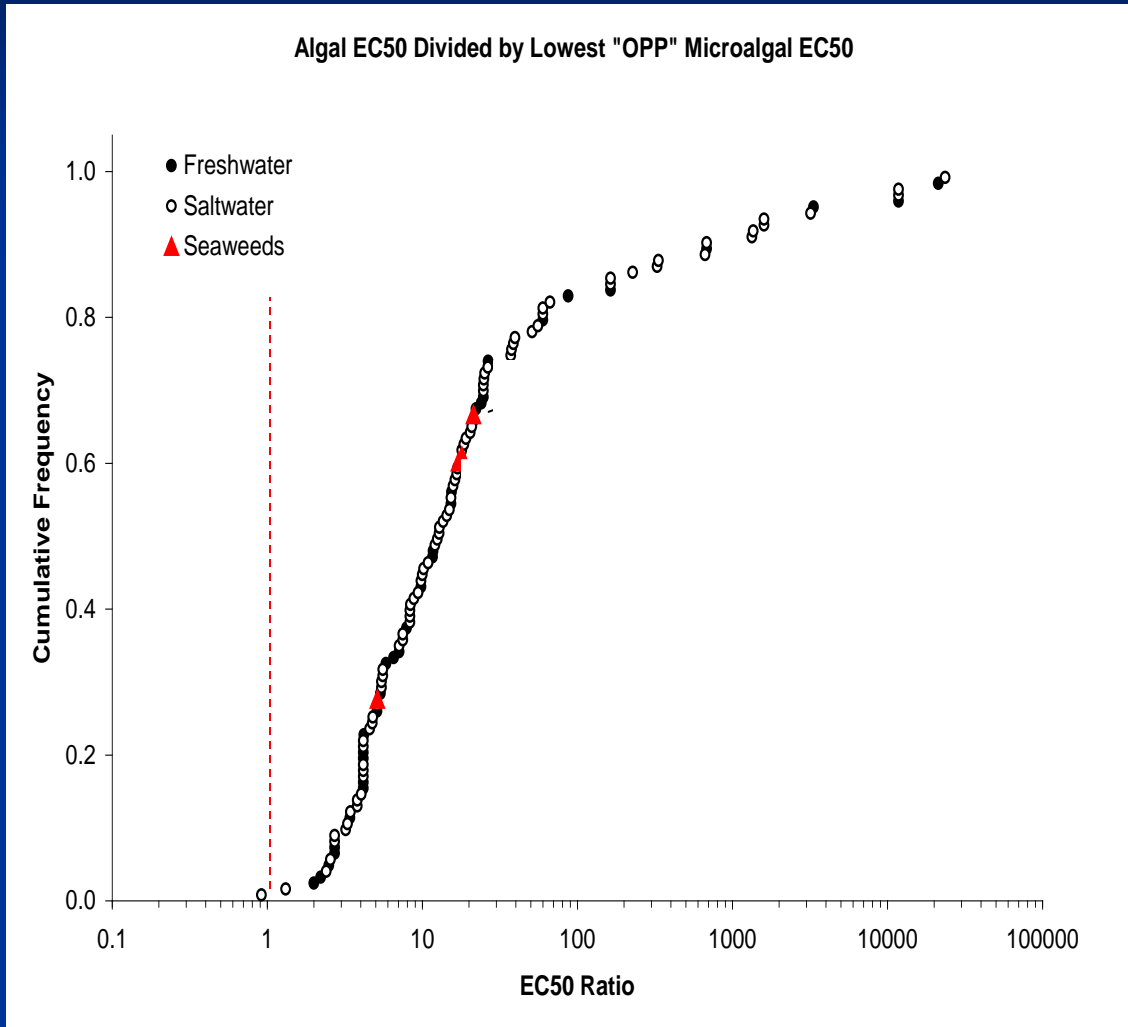


- Most plant studies conducted using freshwater species, especially microalgae

Non-vascular

- Are the sensitivities of current microalgal species representative of non-vascular plant sensitivities?
 - Limited information available for comparison of sensitivities of standard algal species to other non-vascular families such as mosses and liverworts
 - Many tests compared sensitivities of various freshwater microalgal species - great variation (2 to 10-fold differences) between species for same toxicant
 - Sensitivities of freshwater vs. saltwater algae are not well understood

Non-vascular



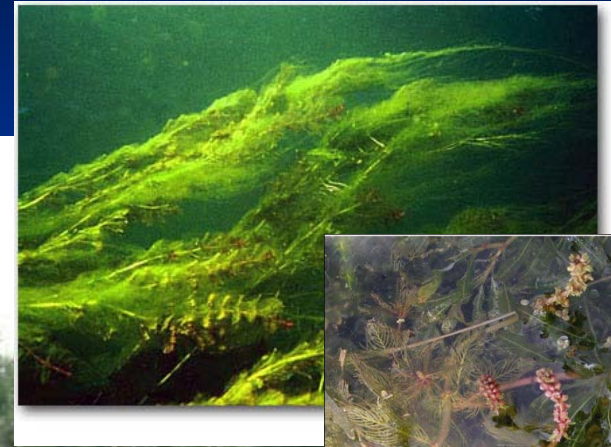
- Ratios of non-vascular species EC50s to lowest OPP microalgal species EC50s

Vascular Plants

Is the sensitivity of *Lemna* representative of vascular plants sensitivity?



Eds. Roy Mennema



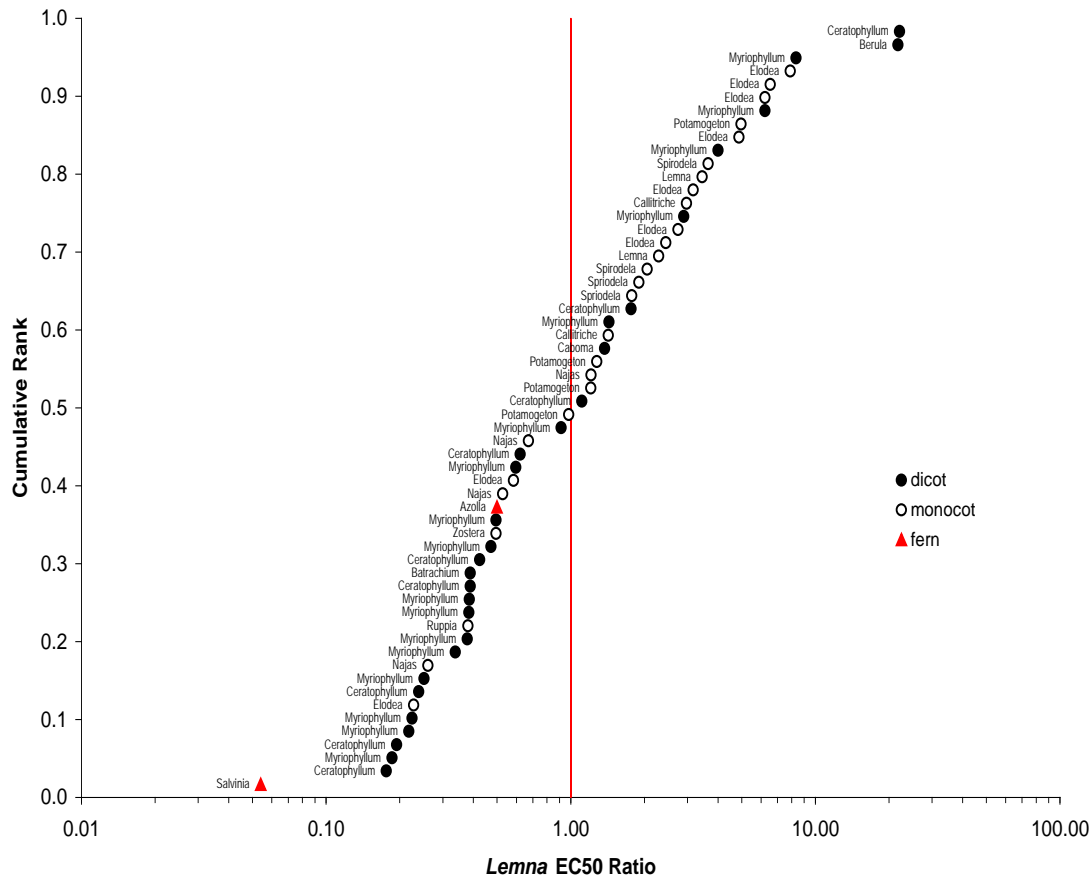
Tape grass
Vallisneria spiralis
Photo by Vic Ramsey
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Vascular Plants



■ Ratios of vascular plant EC50s to *Lemna* EC50s

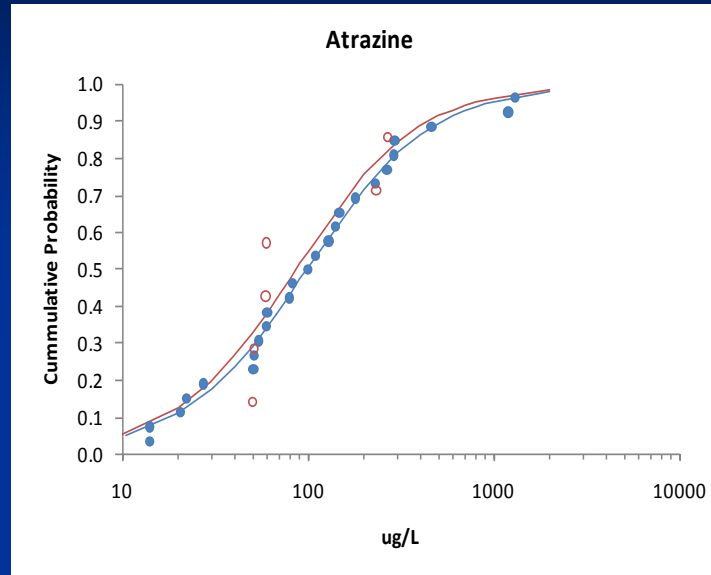
Aquatic Life Screening Values

- Represent a means of reflecting the sensitivity of vulnerable aquatic plant species in an aquatic community
- Derive ALSVs using input gathered from stakeholders
 - Using lowest test value
 - Extrapolation factors
 - Species Sensitivity Distributions (SSDs)
 - QSAR

SSDs

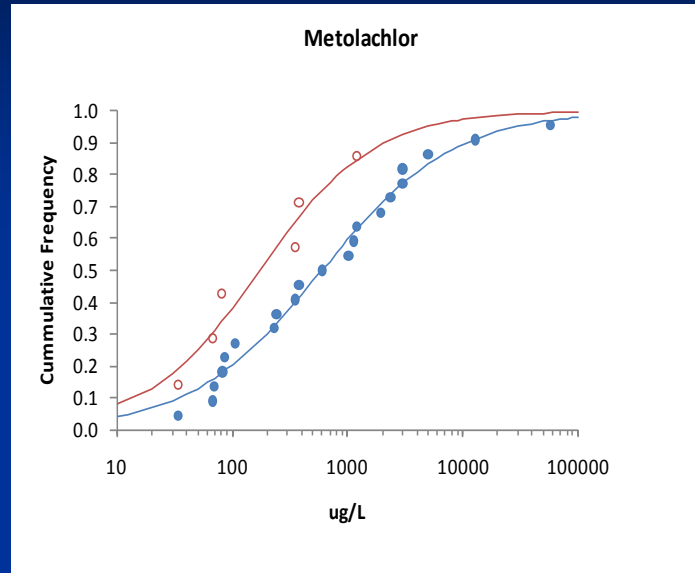
- Data from ECOTOX and draft atrazine document
- Six pesticides with large data sets
- EC50 chosen as effects endpoint
- Benchmark values calculated by non-linear regression and using 1985 Guidelines methods
- Comparison of endpoints derived from robust dataset to less robust dataset (OPP species only)

Example SSDs



Compound	N	Calculated HC ₅		FAV 5 th percentile		Standard OPP data	
		Full data set	Partial Data	Full data set	Partial Data	Lowest EC ₅₀	Species
Atrazine	25	10.37	9.69	13.79	50.52	50.5	<i>Lemna gibba</i>

Example SSDs



Compound	N	Calculated HC ₅		FAV 5 th percentile		Standard OPP data	
		Full data set	Partial Data	Full data set	Partial Data	Lowest EC ₅₀	Species
Metolachlor	21	12.47	6.02	38.88	29.69	34.2	<i>P. subcapitata</i>

SSDs

- Most sensitive OPP species was around the HC₁₀ in most examples
- Most sensitive species was not consistent between chemicals
- Using non-linear regression analysis with full data set was the only method that utilized all available data
- Equation and percentile chosen not necessarily the preferred approach

Proposed Next Steps for Plant ALSV Methods

- No consensus on data set that is representative of aquatic plant community
- Six example chemicals have large enough data sets that are likely robust enough to characterize a variety of sensitivities
- No consensus on appropriate exposure durations or appropriate measurement endpoints

Proposed Next Steps for Plant ALSV Methods

- Expand upon the six SSDs already developed
- Add large datasets that are inclusive of OPP species, maximizing chemicals represented
- Explore development of extrapolation factors that could potentially allow limited datasets to represent protection of the larger aquatic plant community
- Other vascular plants (besides *Lemna*) may be important species needed to develop a robust dataset

Desired Stakeholder Input

- Data sets, unpublished data
- Comments on the most appropriate HCx level to select for the comparison between large data sets and smaller, less diverse data sets
- Comments on the most appropriate sigmoid curve equation to use in non-linear regression of SSDs
- Comments on how to address uncertainties in the data

Summary

- The white paper for aquatic plants provides an overview of plant toxicity data and examples of how the data may be used to derive ALSVs.
- Uncertainties exist in the data (e.g., duration, effects endpoints), which are important to consider when combining into an SSD.
- All approaches need to be further assessed, and the amount of available data should be considered in each case.