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

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**List of Acronyms**

| | |
|---|---|
| ALSV | Aquatic Life Screening Value |
| ALWQC | Aquatic Life Water Quality Criteria |
| APHA | American Public Health Association |
| ASTM | American Society for Testing and Materials |
| CWA | Clean Water Act |
| $EC_x$ | Effective Concentration for x% of response |
| EF | Extrapolation Factor |
| EPA | Environmental Protection Agency |
| EU | European Union |
| FIFRA | Federal Insecticide, Fungicide and Rodenticide Act |
| FAV | Final Acute Value |
| FCV | Final Chronic Value |
| FPV | Final Plant Value |
| GMAV | Genus Mean Acute Value |
| $HC_x$ | Hazardous concentration threshold for x% of organisms |
| $IC_{50}$ | Inhibition concentration for 50% of organisms |
| $LC_{50}$ | Lethal Concentration for 50% of organisms |
| LOC | Level of Concern |
| MATC | Maximum Allowable Toxicant Concentration |
| MPCA | Minnesota Pollution Control Agency |
| NOAEC | No Observed Adverse Effect Concentration |
| NOEC | No Observed Effect Concentration |
| NPDES | National Pollutant Discharge Elimination System |
| OECD | Organization for Economic Cooperation and Development |
| OPP | Office of Pesticide Programs |
| OW | Office of Water |
| SETAC | Society of Environmental Toxicology and Chemistry |
| SMAV | Species Mean Acute Value |
| SSD | Species Sensitivity Distribution |

## 1. Executive Summary

The Environmental Protection Agency (EPA) assesses the impact of pesticides on aquatic plants based on the requirements of the Clean Water Act (CWA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The goal of this project is to build on the existing approaches used by the Office of Pesticide Programs (OPP) and the Office of Water (OW) and to explore tools which both program offices may use to characterize effects to aquatic plant communities with varying amounts of data.

This paper summarizes several different approaches that are currently being used by the EPA, states and regulatory agencies in other countries which account for the uncertainty associated with limited datasets for aquatic plants and the extent to which available data are representative of the entire plant community. These methods may be used to characterize potential adverse effects of pesticides on aquatic plant communities.

There are two major uncertainties associated with aquatic plant toxicity data that should be considered when characterizing effects of chemicals on aquatic plants. The first involves the variability associated with toxicity study endpoints. While there are standardized testing methodologies from regulatory agencies and standard writing organizations, the endpoints available for aquatic plants exposed to the same chemical can vary (*e.g.*, test duration, effect type, summary statistic may differ in each test). The second uncertainty involves the unknown sensitivity of test species to a chemical stressor relative to other aquatic plant species.

The term "aquatic life screening value" (ALSV) is introduced here to represent community-level benchmarks. This paper describes methods that may be considered to develop ALSVs for aquatic plant communities using available toxicity data. These plant ALSVs are considered separately from animal ALSVs. A plant ALSV may simply be based on the lowest available aquatic plant toxicity data.

In cases where there is evidence to suggest that the available toxicity data are not representative (*i.e.,* based on an understanding of the general sensitivity of plants to particular modes of action and whether sensitive species are represented in the dataset) of the most sensitive plant species that are expected to be impacted, extrapolation factors (EFs) could be applied to available data to derive the plant ALSV. Extrapolation factors are set (default) values that are applied to available toxicity data to account for various sources of uncertainty in extrapolating from individual species toxicity data to assessment endpoints. When a larger dataset is available, this paper also demonstrates that using a specified lower confidence interval for the plant species sensitivity distribution (SSD) can provide a plant ALSV. Although this paper broadly describes approaches for deriving aquatic plant ALSVs similar to those under consideration for aquatic animals, it is expected that these approaches will be further refined as EPA reviews available methods.

## 2. Introduction

The definition of "plant" varies among taxonomists. Some taxonomists reserve the term "plant" to represent those organisms in the Kingdom Plantae. All vascular plants and some non-vascular aquatic plants are in the Kingdom Plantae. Among the non-vascular

aquatic plants, free living algae include groups from four separate kingdoms[1], including Bacteria (*e.g.,* cyanobacteria); Protozoa (*e.g.,* euglenoids and dinoflagellates), Chromista (*e.g.,* diatoms) and Plantae (*e.g.,* green algae). For the purpose of this white paper, the term "plant" includes all photosynthetic organisms that contain chlorophyll *a*—which has members in each of the above four Kingdoms.

Aquatic plants have members in every conceivable freshwater and saltwater habitat. Aquatic plants have various growth habits, such as attached (rooted or other holdfast), free-floating, submerged or emergent (only partially submerged for part or all of their life history). Aquatic plants also form the base of most aquatic food chains, are important habitat components of aquatic ecosystems and are functionally important in carbon assimilation and oxygen evolution.

The Environmental Protection Agency (EPA) assesses the impact of pesticides on aquatic plants based on the requirements of the Clean Water Act (CWA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The goal of this project is to build on the existing approaches used by the Office of Pesticide Programs (OPP) and the Office of Water (OW) to explore common approaches that both programs may use to characterize effects to aquatic communities using the best available scientific tools and methodologies with varying amounts of data. In 2010, EPA conducted six regionally-based public meetings and drafted three white papers describing additional tools and approaches that may be used to augment the ability of the EPA, and states, local and tribal water management agencies to derive taxa specific and cross-taxa (community) benchmark values for chemicals such as pesticides. One paper, entitled "*Predicting the Toxicity of Chemicals to Aquatic Animal Species*" (hereafter referred to as the tools white paper), describes potential additional tools that can be used to estimate toxicity data. The tools paper primarily focuses on methods for estimating toxicity of chemicals to aquatic animals, as there are limited tools available to estimate toxicity to aquatic plants. Two other papers describe methodologies to estimate community effects, one on aquatic animals entitled "*Exploration of Methods for Characterizing Effects of Chemical Stressors to Aquatic Animals*" (hereafter referred to as the "aquatic animal white paper") and this paper on aquatic plants. The term "aquatic life screening value" (ALSV) is introduced here to represent community-level benchmarks. This paper describes methods that may be considered to develop ALSVs for aquatic plant communities using available toxicity data for aquatic plants. These plant ALSVs are considered separately from animal ALSVs, which are described in the aquatic animal white paper.

This white paper describes various methodologies used to evaluate effects of chemicals on aquatic plant communities. It explores uncertainties associated with available toxicity tests and their relationships to aquatic plant assessment endpoints. The paper compares the relative sensitivities of the test species for which acceptable toxicity data are available. Finally, this paper provides a conceptual approach that may be used to integrate chemical-specific data, tools and methods (*i.e.,* extrapolation factors and sensitivity distributions) for deriving plant ALSVs that may be used by EPA, States and

[1] Note the arrangement of "life" into taxonomic categories has been in a state of flux ever since Linnaeus first introduced Animale and Vegetabile. The main point here is not to definitively support one particular scheme or another, but to point out that what we traditionally refer to as plants is an extremely diverse group. Kingdom titles used above are based on Cavalier-Smith (2004).

Tribes to interpret aquatic ecological risks associated with chemical exposure information (*e.g.*, monitoring data). The tools and methods discussed in this paper are intended to compensate for limited data in describing potential effects of specific chemicals on aquatic plant communities and would provide regulators with a means of deriving advisory values that will ensure the protection of the aquatic environment.

## 3. Existing methodologies to evaluate plant effects

As described below, there are several different approaches currently used by regulatory agencies to derive measures of effect for aquatic plants based on available data. Measures of effect, such as toxicity test results from a laboratory study, are used to quantitatively represent assessment endpoints. An assessment endpoint is "an explicit expression of the environmental value to be protected."[2]

### 3.1. 1985 Guidelines Method for Deriving ALWQC (OW)

The USEPA, as stated in the CWA, is tasked with establishing criteria values for various pollutants found in the waters of the United States. These criteria serve as guidance for States and Tribes to use in developing their water quality standards. The current OW methodology for deriving criteria is outlined in the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (termed: "the 1985 Guidelines"; USEPA 1985). These OW guidelines focus on deriving criteria that are based on animal toxicity data, and data for plants have been considered more essential recently than in the past. In these guidelines, an acute criterion is derived using acute toxicity data for animals. A chronic criterion is derived using the most sensitive of the final chronic value (FCV) for animals or the final plant value (FPV).

The 1985 Guidelines provide limited guidance on deriving the FPV. The OW minimum data requirements for the FPV are results of one acceptable test with a freshwater alga (non-vascular plant) or vascular plant (for the freshwater criterion) and one acceptable test with a saltwater alga or vascular plant (for the saltwater criterion). At the time that the original guidance was written for derivation of ALWQC, "*procedures for conducting tests with plants and interpreting the results of such tests [were] not well developed*" (USEPA 1985). Because of this, a definitive set of minimum data requirements (including specific genera or families) is not currently required for aquatic plants in OW. The only additional specification is if plants are expected to be more sensitive than animals. In this case, the "results with a plant in another phylum (division) should also be available"; however, the second test result is not required.

In addition, the types of test procedures are vaguely described in the 1985 Guidelines. "*A plant value is the result of a 96-hr test conducted with an alga or a chronic test conducted with an aquatic vascular plant.*" The guidelines describe the calculation of the FPV as a number that "*should be obtained by selecting the lowest result from an*

[2] http://www.epa.gov/OCEPATERMS/

*acceptable test with an important aquatic plant species in which the concentrations of test material were measured and the endpoint was biologically important*." The definitions, however, of "*important aquatic plants*" or "*biologically important*" endpoints are not given.

Plant toxicity data available for use in a criterion derivation are frequently absent, and no criteria to date have been published that use plant data in the calculation. The guidelines state that a criterion that is protective of aquatic animals will "*probably protect aquatic plants*." This assumption, however, clearly has not held true for herbicides (such as atrazine, acetochlor and metolachlor) and cannot be assumed for other pesticides or even chemical pollutants in general (Lewis 1990, 1995; Wang and Freemark 1995). **Table 1** lists a variety of chemical classes for which data have shown plants to be more sensitive than animals.

**Table 1. Examples of contaminants found more toxic to algae than animals[a].**

| | |
|---|---|
| **Trace Metals** | **Wastewaters** |
| Cadmium | Paper Mill effluent |
| Copper | Textile effluent |
| Nickel | Oil Refinery effluent |
| Zinc | |
| **Alcohols** | **Organics** |
| Butanol | Acridine |
| Diethylene glycol | Acrylates |
| Heptanol | Chloramine |
| Hexanol | Chloroacetaldehyde |
| Isooctanol | Chloronapthalene |
| Octanol | 4-Chlorophenol |
| Proporgyl alcohol | Dibenzofuran |
| **Pesticides (*Herbicides)** | 1,3- Dichlorpropene |
| Aldrin | Dinitrotoluene |
| Atrazine* | Nitrobenzene |
| Chlordane | 4-Nitrophenol |
| 2,4D* | Organotin |
| Dieldrin | Phenol |
| Diquat* | Potassium chlorate |
| Endrin | Potassium dichromate |
| Glyphosate* | Sodium fluoride |
| Tebuthiuron* | Sodium tetraborate |
| **Surfactants** | 2,4,6-Trinitrophenol |
| Ditallow dimethyl ammonium chloride | |
| Trimethyl ammonium chloride | |
| Sodium dodecyl sulfate | |

[a]Adapted from review table in Lewis (1995).

### 3.2. Draft Atrazine Aquatic Life Criteria

The Office of Water is currently deriving an Aquatic Life Water Quality Criteria (ALWQC) for the herbicide atrazine[3]. This represents the first time that an ALWQC will be developed where plant data are used to establish chronic protection limits.

In the draft ALWQC, separate freshwater and saltwater FPVs are derived. The saltwater FPV is based on the lowest species mean acute value (SMAV) for sago pondweed (*Potamogeton pectinatus*[4]). For the freshwater FPV, a unique method is being considered that uses a large number of laboratory toxicity studies and experimental ecosystem studies (Erickson 2010). In this method, original data were used to create dose-response curves for each species based on a common endpoint. Rather than using a species sensitivity distribution (SSD) consisting of $EC_{50}$ values alone, the method results in a distribution of dose response curves for individual plant species. The experimental designs of the mesocosm studies varied in concentrations tested, duration of exposures and effect level observed. The data from these experiments provide information on effects to actual plant communities which can be used to determine what magnitude of effect on the species composition of the aquatic plant community. It should be noted that the methods used for the atrazine ALWQC are unique to atrazine because they rely upon a large aquatic plant toxicity database (that includes both single species toxicity testing as well as numerous mesocosm studies) that is available for this chemical.

### 3.3. Minnesota Standards for Acetochlor and Metolachlor

States have explored how aquatic plant toxicity data could be used to develop water quality standards since herbicides are frequently detected in US surface waters and aquatic plants are an obvious susceptible taxonomic group for these chemical stressors. To derive the state water quality standards for acetochlor and metolachlor, the Minnesota Pollution Control Agency (MPCA) analyzed available aquatic plant and animal toxicity data for each herbicide. As might have been expected, aquatic plants were more sensitive than animals to both herbicides. Consistent with the Great Lakes Initiative (GLI) Tier II (USEPA 1995) approach, the acute regulatory endpoints for these compounds were calculated using the available animal data and plant data were considered to represent chronic effects.

The minimum data requirements for the acute criterion (eight animal families) were not available to derive the acute value using the 1985 guidelines, thus the acute values for both herbicides were derived using the GLI Tier II methodology (USEPA 1995). This methodology is described in the aquatic animal white paper.

Chronic standards for acetochlor and metolachlor were developed using only plant data from the OPP database and the open literature. In deriving these criteria, the MPCA had

[3] June 23, 2009 draft document prepared by Great Lakes Environmental Center and University of Wisconsin-Superior.
[4] Now called *Stuckenia pectinatus*. www.plants.usda.gov

goals of protecting the integrity of the plant community, protecting sensitive plant species and achieving a 20th percentile level of protection. The MPCA derived species sensitivity distributions for each chemical using geometric means of $EC_{50}$ values for the same species (if more than one value was available for a species). The Minnesota chronic standard for metolachlor is 23 µg/L to protect coon's tail (*Ceratophyllum demersum*), a resident aquatic vascular plant. This value is lower than the 5th percentile of the $EC_{50}$ distribution for aquatic plants (36 µg/L). Lowering the standard to protect an important species, such as the coon's tail is similar to the provision set out in the 1985 guidelines that allows for lowering the criterion to protect commercially or recreationally important species. The Minnesota chronic standard for acetochlor is 3.6 µg/L, which is consistent with the 20th percentile of the distribution of $EC_{50}$ values for aquatic plants.

After the above standards were proposed, some reviewers commented on some challenges faced by MPCA in deriving the chronic acetochlor criterion. One criticism of the criterion was the separation of $EC_{50}$ and MATC[5] values into separate distributions. In a review of MPCA's methodologies, an independent researcher used the dose-response raw data from the individual plant studies to calculate an $EC_{20}$ value for all studies. All $EC_{20}$ values were plotted in one distribution, increasing the number of studies used to derive a criterion from the 20th percentile value[6]. Also included in the review of MPCA's methodology, was a standardization of the measured endpoint and exposure duration. While MPCA used a variety of growth endpoints (e.g., dry weight, frond number, cell density) and exposure durations, the reviewer's method limited data to include only four day algae studies measuring cell density, four or seven day duckweed studies measuring frond number and seven day rooted macrophyte studies measuring dry weight. This is consistent with the recommendation from the Society of Environmental Toxicology and Chemistry (SETAC) Europe Workshop on Aquatic Macrophyte Risk Assessment for Pesticides that, where practical, SSDs should be created using similar protocols and endpoints (Maltby et al. 2010).

### 3.4 University of California-Davis Methodology

In the UC-Davis Methodology (TenBrook *et al*. 2010), herbicides must be evaluated using data from algae or vascular plants. Only chronic criteria are derived for plants. For herbicides, and for pesticides where plants are the most sensitive taxa, plant-only SSDs should be used, provided there are reliable and relevant studies for five different plant species. If there are fewer than five plant species, the criterion is determined as the lowest plant NOEC with a relevant endpoint, similar to the 1985 Guidelines final plant value.

### 3.5. Ecological Effects Characterization and Benchmark Derivation (OPP)

OPP, under FIFRA, has the authority to require data in support of the registration of a pesticide product. Accordingly, OPP has developed regulations (40 CFR Part 158[7])

[5] MATC =Maximum Allowable Toxic Concentration. It is calculated as the geometric mean of the lowest observable effect concentration and the no observed effect concentration.

[6] Giddings, JM. 2007. Review of Plant-Based Acetochlor Class 2 Water Quality Standards for Minnesota.

[7] Code of Federal Regulations. 2010. http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=1a3c043b5425ffaa607dd0f14f9bddbb&tpl=/ecfrbrowse/Title40/40cfr158_main_02.tpl

which specify the types and amount of information that pesticide companies must routinely submit to EPA to support product registration. Section 158.660[8] describes the plant protection data requirements and specifies the type and amount of data the Agency needs to characterize the effects of a pesticide on aquatic plants and is based on proposed or existing use(s) (how and where the pesticide is applied). As with animal testing requirements, OPP relies on a tiered approach for examining effects to aquatic plants. The Ecological Effects Test Guidelines (Sub-part G of 40 CFR Part 158) describe three testing tiers for assessing the effects of pesticides on non-target aquatic plants. The first tier assesses the effect on plant growth resulting from a single test concentration equivalent to the estimated environmental concentration resulting from the maximum labeled application rate or a limit concentration. At minimum, Tier I tests are required for all pesticides. If Tier I testing shows growth reduction or visual phytotoxicity of >50% inhibition, or the Tier I test does not provide a definitive no observable adverse effects concentration (NOAEC), Tier II tests are required. Tier II tests involve multiple test concentrations and are intended to generate $EC_{50}$ and NOAEC values for the test species which exhibit detrimental effects in the Tier I testing. While Tier I and II tests are laboratory tests, Tier III tests are field studies which are designed to evaluate adverse effects during critical stages of development on sensitive native plant communities. To date, Tier III aquatic plant tests have been submitted to OPP for only a few compounds (*e.g.*, diquat, irgarol).

OPP uses a screening approach to assess plant sensitivity to pesticides, which relies on a suite of toxicity tests performed on a specified set of surrogate species. For aquatic plants, these categories are based on the presence or absence of a vascular system. For non-vascular aquatic plants, the surrogate species generally are *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum;* green alga), *Anabaena flos-aquae* (cyanobacterium), *Navicula pelliculosa* (pennate freshwater diatom) and *Skeletonema costatum* (centric marine diatom). *Lemna* species (usually either *L. gibba* or *L. minor*) serve as surrogates for aquatic vascular plant species (USEPA 2004). Specific guidelines are available for the tiered testing of aquatic non-vascular and vascular plants. Guidelines 850.5400[9] and 850.4400[10] describe the test procedures and conditions for microalgae and aquatic vascular plants, respectively. Test guideline 850.4450[11] pertains to the test procedures and conditions for field, microcosm, and mesocosm studies that cover a broader range of plant types and study durations (tests typically continue for the entire life cycle of the test plant). If data are available for other, non-standard, non-vascular or vascular species (*e.g.*, from the scientific literature) that show greater sensitivity than those results from the standard test species, OPP uses data for the more sensitive species in risk assessments provided those studies meet acceptability criteria. Endpoints suitable for quantitative use for all species are used in the risk characterization.

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[8] http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=1a3c043b5425ffaa607dd0f14f9bddbb&rgn=div8&view=text&node=40:23.0.1.1.9.7.1.2&idno=40

[9] http://www.epa.gov/ocspp/pubs/frs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-5400.pdf

[10] http://www.epa.gov/ocspp/pubs/frs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-4400.pdf

[11] http://www.epa.gov/ocspp/pubs/frs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-4450.pdf

OPP's measurement endpoints for aquatic non-vascular plants focus primarily on algal growth rates and biomass (vegetative reproduction) measurements based on 4-day (96-hr) tests. The most sensitive $EC_{50}$ value of the four non-vascular plants (no distinction is made between freshwater and marine plants) is typically used to characterize effects to aquatic plants and in risk assessment. Measurements on vascular plants typically include growth (frond number at Day 0, 3, 5, and 7 of exposure), growth rate, mortality, biomass, measurements at the end of the 7-day test. Again, OPP typically selects the most sensitive $EC_{50}$ for the vascular aquatic plant endpoint.

The OPP benchmarks were developed in response to recommendations and input from stakeholders, who were concerned about potential effects of pesticides with no existing ALQWC. OPP developed a webpage of non-regulatory taxa-specific aquatic toxicity endpoints referred to as "OPP Aquatic Benchmarks"[12]. These Benchmarks are based on the most sensitive toxicity data (considering registrant-submitted studies and the scientific literature) from OPP's ecological risk assessments of specific pesticides. For non-vascular and vascular plants, benchmarks are calculated by multiplying each lowest $EC_{50}$ value by the plant Level of Concern (LOC = 1.0), which is based on OPP's ecological risk assessment process. LOCs are the Agency's interpretative policy and are used to analyze potential risk to non-target organisms and the need to consider regulatory action. LOCs are used to indicate when a pesticide use (as directed on the label) has the potential to cause adverse effects on non-target organisms (USEPA 2004).

### 3.6. Current International Methods for Incorporating Aquatic Plant Data into Aquatic Life Guidelines

Throughout the world, aquatic life guidelines for contaminants are derived using different methodologies and data requirements. The use of plant data is not always required, and in some cases plant data are only required for herbicides or phytotoxic compounds. For instance, the province of Quebec does not require plant data (MENVIQ 1990), while the Canadian Water Quality Guidelines for the Protection of Aquatic Life Protocol (CCME 2007) requires plant data for their two highest tier guidelines, but not the lowest tier guideline. The European Union (EU) requires algal data as part of their "base set" of test values, and the algal data are incorporated when the species sensitivity distribution (SSD) approach is used to derive the guideline, while plant data will reduce the extrapolation factor (EF), when an SSD is not used (European Commission 2003). The EFs are used in the risk assessment process to account for uncertainty in the data.

In other countries, the type of chemical being assessed for guideline derivation may play a role in the amount of plant data required. Canada requires only one plant study for the two highest tier guidelines, unless plants are the most sensitive (*e.g.*, with herbicides), in which case three plant toxicity values are required. For the lowest tier guideline, no plant data are required unless the plants are the most sensitive organisms (CCME 2007). The EU also requires additional plant studies when the stressor of concern is an herbicide or plant growth regulator (European Commission 2003).

[12] OPP Aquatic Benchmark Table. Available online at: http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm

When both plant and animal data are required in deriving a guideline for a contaminant, most countries assess the plant toxicity values alongside the toxicity values for animals. Canada and the EU include plant toxicity values along with animal values in their SSDs when deriving their highest tier guidelines. Canada generally considers most standardized plant tests to be chronic data, while the EU considers algal data to be chronic if the endpoint is a NOEC rather than an $EC_{50}$ (CCME 2007, European Commission 2003). For most countries using EFs, they are applied to the lowest toxicity value, whether it be plant or animal. EFs vary depending on the amount of toxicity data that are available and depending on whether an acute or chronic guideline is being derived. For chronic toxicity values, Canada and the EU apply an EF of 10 to the lowest toxicity value (plant or animal) to derive their regulatory endpoint. However, the required EF is higher in EU if plant data were not available in the analysis.

## 4. Uncertainties Associated with Aquatic Plant Toxicity Data

There are two major uncertainties associated with aquatic plant toxicity data that should be considered when characterizing effects of chemicals on aquatic plants. The first involves the variability associated with endpoints resulting from toxicity studies. The second involves the unknown sensitivity of test species to a chemical stressor relative to other untested aquatic plant species. These uncertainties are described below.

### 4.1. Variability Associated with Endpoints

A variety of aquatic phytotoxicity test methods (laboratory and *in situ*) have been described in the scientific literature during the last 40 years for various single species of freshwater and saltwater algae (mostly microalgae), vascular plants, periphyton and phytoplankton assemblages. These include the laboratory methods described by standard writing organizations and regulatory agencies such as American Public Health Association (APHA Standard Methods for the Examination of Water and Wastewater), Organization for Economic Cooperation and Development (OECD Guidelines for Testing of Chemicals), American Society for Testing and Materials (ATSM Standards on Biological Effects and Environmental Fate) and USEPA (850 series – OCSPP Harmonized Test Guidelines and National Pollution Discharge Elimination System [NPDES] Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters).

Despite the availability of standard methods, the measurement endpoints available for aquatic plants exposed to the same chemical can vary. Plant sublethal effects extend to a variety of attributes which include biomass (*e.g.*, cell count, growth rate, pigment content), activity (*e.g.*, fluorescence, oxygen evolution, ethylene production) and biochemical attributes (*e.g.*, ATP level, enzyme activities). In practice, the receptor attributes most often used by OPP and OW are either some aspect of growth or growth rate. Biochemical effects can be early warning indicators of environmental stressors as toxicity in aquatic plants is first manifested at the biochemical level before effects are evident at the whole-organism level. Activity or biochemical attributes are generally not

routinely considered by the EPA, unless a peer reviewed quantitative relationship can be established between the biochemical measurement endpoint and the Agency's apical assessment endpoints. The sublethal biochemical effects that are commonly reported in aquatic plant studies in response to stressors include changes in carbon fixation, plant pigments, carbohydrate content, cytochrome F, ethylene/ethane, oxidative enzyme activity and protein concentration, enzyme levels, antioxidant levels, formation of stress proteins, chlorophyll fluorescence and lipid peroxidation. These effects often may be more sensitive, but their environmental relevance, relationship with more standard endpoints, and link to whole plant effects is not known and/or poorly characterized. Like the situation with animals, the connection between these three desired assessment endpoints and some of the non-traditional measurement endpoints has not been well established. Reproduction endpoints with aquatic plants have generally been restricted to vegetative reproduction. Sexual reproduction has received much less attention, but may be a more sensitive plant endpoint as compared to vegetative growth. For instance, the $EC_{50}$ value for reproduction in the marine red alga *Ceramium strictum* when exposed to phenol is 5,000 µg/L, while the growth $EC_{50}$ value is 10,000 µg/L (Eklund 1998, Bruno and Eklund 2003). Therefore, more sensitive endpoints may not always be captured in typical studies that evaluate vegetative growth.

Plant summary statistics are often expressed as either a no observable adverse effect concentration (NOAEC), $EC_{50}$ or some other $EC_x$ level. When an EC endpoint is selected other than $EC_{50}$ it is often chosen in an attempt to provide a substitute for an NOAEC (*e.g.*, $EC_{20}$). This is usually done to eliminate some of the concerns about using hypothesis testing in establishing no effect concentrations when the study has a regression-based design and inadequate replication to support hypothesis testing. Concerns with the NOAEC include the following: 1) the potential for a biologically significant effect at a test concentration where observed effects were not statistically significant; 2) the NOAEC is heavily influenced by the test concentrations that were chosen for the test; and 3) confidence intervals cannot be derived (Crane and Newman 2000).

The exposure durations of standardized tests often differ, which brings up the consideration of whether plant tests of different exposure durations are appropriate to combine in deriving screening values or ALWQC. Most microalgal tests have exposure durations of either 72 or 96 h; *Lemna* often range between 4 and 7 d; durations for toxicity tests conducted with rooted vascular plants (freshwater) have ranged from 1 hr to 6 wk (Lewis and Wang 1999); test durations with seagrasses have been conducted between 2 hr and 42 d (Lewis and Devereux 2007) and between 1 and 26 wk for mangroves (Lewis *et al*. in review). If growth rate is the receptor attribute of interest then data from tests using different exposure durations could probably be combined (as is being proposed for a portion of the draft atrazine ALWQC). However, if pesticide exposure concentrations are not measured (which is often the case with plant tests), then care should be taken when including data from widely differing exposure durations—the actual exposure concentrations could be vastly different. Rentz and Hanson (2009) merged data from multiple exposure durations from tests with aquatic macrophytes by adjusting them all to the same duration. They used Haber's rule which may not have been

appropriate. While Haber recognized that C x t = k [where C = concentration, t = time, k = constant] was applicable only under certain conditions, many toxicologists have used this rule to analyze experimental data whether or not their chemicals, biological endpoints, and exposure scenarios were suitable candidates for the rule. Haber studied the acute lethality of war gases and his exposure durations were on the order of a few minutes to several hours. There is no indication that he advocated his rule held for all toxins and for exposures ranging from days to weeks (Miller *et al*. 2000).

OPP's guidance suggests using the most sensitive measurement endpoint as long as it can be related to the assessment endpoints of survival, growth or reproduction. This generally includes 96-h $EC_{50}$ values for non-vascular plants and 7-d $EC_{50}$ values for vascular plants based on biomass or growth rate. When considering available data for aquatic plants exposed to a chemical stressor, an attempt should be made to make the endpoints as consistent as possible. For instance, endpoints with similar durations and measures of effect (*e.g*., $EC_{50}$) should be considered together. This will ensure that the variability in responses to stressors can be attributed to differences in species responses, rather than variability in endpoints.

### 4.2. Relative Sensitivity of Test Species to other Aquatic Plants

The ECOTOX database[13], which is maintained by the EPA's Office of Research and Development Mid-Continental Ecology Division (ORD/MED) is a source of open literature ecological effects data on single chemicals for aquatic and terrestrial plants and animals. Based on the results of 2008 query of ECOTOX, approximately 23,000 studies were conducted with aquatic plants and of these, the majority (86%; 19,700 entries) were with freshwater species. The majority of the aquatic plant studies (18,700) were conducted with microalgae. While 2,120 were conducted with duckweeds, 570 with submerged grasses, 4 tests with mangroves and about 1,670 tests with other vascular plants (**Figure 1**). Only a relatively small number of tests (1,072 studies) have been conducted on pesticides – the majority of plant studies were conducted with metals or other toxicants such as high production volume chemicals. It should be noted that the ECOTOX database contains aquatic plant toxicity data submitted to OPP by pesticide registrants.

[13] Available online at: http://cfpub.epa.gov/ecotox/

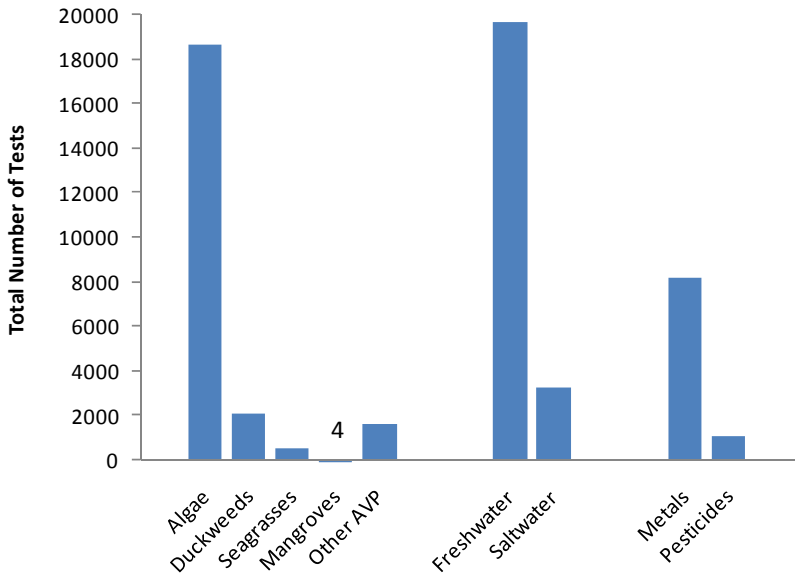


**Figure 1. Summary of available aquatic plant data from USEPA's ECOTOX database as of 2008. Other VP means Other Vascular Plants**

As indicated previously, OPP typically receives aquatic plant toxicity data for macroalgae and *Lemna* sp. The relative sensitivities of these test species to other aquatic plants are generally unknown. This can be attributed to limited data for other species of plants exposed to pesticides (**Figure 1**). Section 5 below explores the relative sensitivities of OPP's typical test species with other test species that may be available for the same chemical.

Differences in sensitivities of test species to chemicals may be attributed to the mode of action of the chemical and a plant's physiology. For instance, since herbicides often target various aspects of the photosynthetic pathway, there could be differences in response between vascular plants that have C3 vs. those with C4 pathways[14]. For example, *Lemna* species are C3 plants (Longstreth 1989), many saltmarsh grasses (*e.g.*, species of *Spartina* and *Distichlis*) are C4 plants, other saltmarsh plants (*e.g*, species of *Salicornia* and *Scirpus*) are C3 (Drake 1989). In some species of amphibious freshwater plants (*e.g.*, the sedges *Elocharis vivipara* and *E. baldwinii*), the emergent phase exhibits C4 photosynthesis while the submerged phase is C3 (Cronk and Fennessy 2001).

In addition, there is a wide variety of photosynthetic pigments among the various algal groups, so type of accessory pigments (and thus different pigment synthesis pathways) also might make a difference in sensitivity. Although the analysis presented below in this paper did not show any obvious pattern in plant sensitivity relative to major taxonomic groups (*e.g.*, diatoms vs. green algae).

Also aquatic plants have highly diverse habits (*i.e.*, floating, submerged and emergent species) which may need to be considered as the route of exposure varies among these

[14] C3 and C4 refer to the first stable carbon compound in $CO_2$ fixation. In C3 plants this compound is 3-phosphoglyceric acid (a 3 carbon compound) and in C4 plants it is oxaloacetic acid (a 4 carbon compound).

species. The typical vascular plant test species, *Lemna*, is a floating plant, with roots suspended in the water column. This route of exposure (*i.e*., via the suspended roots and undersurface portion of leaves) may not accurately characterize submerged or emergent aquatic plant species, as those that are rooted in the sediment. There currently is a test protocol for the rooted freshwater macrophyte *Myriophyllum* spp. undergoing evaluation for standardization in Europe (Maltby *et al*. 2010).

## 5. Relative Sensitivity of Standard Suite of Aquatic Plant Test Species

In an ideal situation, the sensitivities of a wide variety of aquatic plant groups would be available, represented using a standard set of study durations and measurement endpoints for any chemical for which a plant community toxicity benchmark is desired. Unfortunately, these data sets do not exist for the majority of pesticides of interest. When only minimal data sets are available they usually represent standardized test results because the tests were usually run for regulatory purposes. In practice, however, data from any "reasonable" test procedure are often included in aquatic plant sensitivity distributions (such as in the acetochlor and metolochlor standards in Minnesota). The need to characterize the range of sensitivities in the aquatic plant community may outweigh the desire for comparable test conditions. For example, recent state standards for aquatic plants derived for acetochlor and metolachlor by the Minnesota Pollution Control Agency included freshwater $EC_{50}$ data for algae that had exposure durations ranging from 3 to 21 days and measurement endpoints that included, among others, chlorophyll, abundance, biomass and growth rate. Aquatic vascular plant data for these compounds included exposure durations from 4 to 70 days for endpoints including growth, abundance and dry weight. In addition, the draft atrazine document currently being developed by EPA's Office of Water includes data for freshwater and saltwater algae with exposure durations ranging from 2 to 10 days, and durations of 7 to 35 days for aquatic vascular plants. Endpoints used in the atrazine analyses include population growth rate (*Lemna* spp.), photosynthesis, biomass, chlorophyll, *etc*. Chronic toxicity data for OW's ALWQC can include results derived for different durations since early life stage, partial life cycle, and full life cycle data are equally acceptable. In addition, survival, growth and reproduction endpoints are all acceptable. Combining different exposure durations and endpoints is not unique to hazard analysis for aquatic plants, however, it should be emphasized that it is not ideal either. Future examination of the uncertainty associated with combining durations and endpoints is warranted.

Aquatic phytotoxicity information available to the Agency is primarily that for short-term growth effects for a few single species of easily cultured microalgae and a single floating macrophyte (duckweed). This database may not be sufficiently comprehensive to capture the variability of chemical sensitivities for taxonomically diverse aquatic flora inhabiting different freshwater and saltwater ecosystems. It has been assumed that this predominately freshwater algal-duckweed toxicity data base can serve as an ecologically-relevant surrogate for the sensitivities of the many types of non-vascular and vascular plants, freshwater and marine. Its ability to serve this purpose, however, has not been adequately addressed by the scientific community[15].

[15] Although recently a SETAC advisory group has been formed (Aquatic Macrophyte Ecotoxicology Group) that will address the issue as related to aquatic vascular plants.

The data describing aquatic plant sensitivity to pesticides which is typically submitted by registrants to OPP often includes both $EC_{50}$ and NOEC values. Submitted data from registrants can include those from standardized tests for the four species of microalgae and duckweed (described above in Section 3.5), as well as other aquatic plant species. For convenience, in this paper the standard tests species are referred to collectively as the "OPP standard species" and all others as "non-standard species". It is important to note that, when available, OPP assessments also include non-standard test species data from the open literature that meet quality standards (which is often the case for most herbicides). The microalgal data are intended to represent non-vascular aquatic plants, and the duckweed data are intended to represent vascular aquatic plants. If these are the only data available, one of the obvious key issues is the extent to which these required data are representative of the sensitivity of communities of aquatic plants. Another way to view the issue is to consider how well a benchmark derived using a minimum data set represents a benchmark based on a data set containing a much larger number of data values representing more species of the aquatic plant community. In this latter respect the focus is not on the actual relative sensitivity of various species; otherwise, the effort would need to be restricted to comparisons of paired values using the same duration of exposure and the same measurement endpoint. Rather, the focus here is the relevance of the conclusion for a benchmark derived based largely on a minimal data set as compared to the conclusion about that benchmark if a more diverse database had been available. In this respect it is necessary to make comparisons between toxicity data points that are derived from any reasonably acceptable endpoint—no matter what the relative exposure durations or actual measured endpoints. The analyses below explore this issue in two ways. First, they consider how likely is it to find phytotoxicity values that are less than those values determined for OPP's minimum required species. This is done separately for microalgae (non-vascular) and aquatic vascular plants. Second, they consider the representativeness of a species sensitivity distribution (SSD) using a minimal data set from a distribution based on a more diverse data set

### 5.1. Ratios of OPP standard species endpoints to non-standard species endpoints

The OPP standard test species are selected as the likely minimum data set since any pesticide registered or re-registered in the United States should have these data (especially if a phytotoxic compound is involved). Therefore, not much is to be gained by addressing the issue of having less than these data. As described in the evaluation below, data were normalized by dividing by the data point for the most sensitive OPP standard species in order to be able to combine data from all compounds in one analysis.

#### 5.1.1. Non-vascular species

To explore the uncertainty related to the relative sensitivities of algae, studies that tested various algal species were evaluated using OPP's Ecotoxicity Database of available toxicity data submitted as part of the pesticide registration process. This summary database includes test results from both the OPP standard species and non-standard species. Although this may not include all available aquatic plant pesticide toxicity data,

it provides a comparison of the relative sensitivity of the standard OPP microalgal species with other species of non-vascular aquatic plants for which toxicity data are available. A subset of the microalgal data was used to evaluate this relative sensitivity. Only $EC_{50}$ values were used, but exposure durations ranged from 2 to 10 days. The durations for the OPP standard test species were usually 4 or 5 days. The measurement endpoints used were not listed in the database.

For a pesticide to be included in the evaluation, an $EC_{50}$ had to be available for at least one of the OPP standard species and at least one non-standard algal species. For each chemical included, the $EC_{50}$ value for each non-standard species was divided by the lowest $EC_{50}$ from the data available for OPP standard species. **Table 2** lists the 17 chemicals included in the evaluation along with the most sensitive OPP standard species for each chemical. All but two of the chemicals were herbicides. There was no single species that was consistently the most sensitive to the range of compounds tested. The total number of non-standard species ratios also is included for each chemical[16]. **Table 3** lists the non-standard species for which data were included and the total number of values included in the evaluation for each species. There were 30 species (20 marine and 10 freshwater) listed from 122 tests. Most of the species represented were either green algae or diatoms (10 species each). Data for four of the algal species (all marine macroalgae) came from Di Landa *et al*. (2009), and are not included in OPP's database.

The cumulative frequency distribution for the $EC_{50}$ ratios represented by the species in **Table 3** is graphed in **Figure 2**. Only one ratio is less than 1.0, the ratio of 0.92 for *Isochrysis galbana* exposed to atrazine for 5 days, indicating that the most sensitive of the OPP standard species results may be a sufficient screening value to represent the expected toxicity of a pesticide to saltwater and freshwater algae. Although this conclusion seems to be based on a large database, it is dominated by four non-standard species, *Chlorococcum* sp. (a freshwater green alga), *Dunaliella tertiolecta* (a saltwater green alga), *Isochrysis galbana* (a saltwater golden brown alga) and *Phaeodactylum tricornutum* (a saltwater pennate diatom). There is no apparent pattern to the sensitivity of these species relative to that for the OPP standard species.

[16] If more than one test was available for a non-standard species, then each was included as a separate ratio.

**Table 2. Chemicals selected from the OPP Ecotoxicity Database from which ratios of $EC_{50}$ values for non-vascular plants were calculated[a].**

| Chemical | Most sensitive OPP species | N |
|---|---|---|
| 2,4-D acid | *Skeletonema costatum* | 4 |
| 2,4-D butoxyethanol ester | *Anabaena flos-aquae* | 4 |
| Ametryn | *Pseudokirchneriella subcapitata*[b] | 18 |
| Atrazine | *Skeletonema costatum* | 25 |
| Captan | *Skeletonema costatum* | 5 |
| Dichlobenil | *Navicula pelliculosa* | 4 |
| Diquat dibromide | *Pseudokirchneriella subcapitata* | 4 |
| Diuron | *Pseudokirchneriella subcapitata* | 15 |
| Endothall dipotassium salt | *Anabaena flos-aquae* | 4 |
| Fluometuron | *Anabaena flos-aquae* | 1 |
| Irgarol | *Navicula pelliculosa* | 12 |
| Paraquat dichloride | *Anabaena flos-aquae* | 4 |
| Pentachlorophenol | *Skeletonema costatum* | 2 |
| Prometon | *Pseudokirchneriella subcapitata* | 8 |
| Simazine | *Navicula pelliculosa* | 4 |
| Triallate | *Pseudokirchneriella subcapitata* | 4 |
| Trifluralin | *Navicula pelliculosa* | 4 |
| | Total | 122 |

[a]The denominator of the ratio was the lowest $EC_{50}$ from the available standard species. The most sensitive of these latter species is also listed along with the total number of available $EC_{50}$ values for non-standard species. All chemicals except captan (fungicide) and irgarol (microbiocide) are herbicides. N is the number test results for non-standard species.

[b]Formerly *Selenastrum capricornutum*

**Table 3. List of the non-standard algal species used to calculate the $EC_{50}$ ratios with the most sensitive OPP standard species[a].**

| Species | N | Aquatic Plant Type | Medium |
|---|---|---|---|
| *Chaetoceros gracilis* | 1 | Diatom | Saltwater |
| *Chlamydomonas sp.* | 2 | Green | Freshwater |
| *Chlorella sp.* | 3 | Green | Freshwater |
| *Chlorella pyrenoidosa* | 1 | Green | Freshwater |
| *Chlorococcum sp.* | 16 | Green | Freshwater |
| *Closterium ehrenbergii* | 1 | Desmid | Freshwater |
| *Cyclotella nana* | 1 | Diatom | Saltwater |
| *Dunaliella tertiolecta* | 21 | Green | Saltwater |
| *Eisenia bicyclis* | 1 | Macroalga-Kelp* | Saltwater |
| *Entermorpha intestinalis* | 1 | Macroalga-Green* | Saltwater |
| *Gracilaria tenistipitata* | 1 | Macroalga-Red* | Saltwater |
| *Isochrysis galbana* | 20 | Haptophyte | Saltwater |
| *Microcystis aeruginosa* | 1 | Cyanobacteria | Freshwater |
| *Navicula incerta* | 3 | Diatom | Saltwater |
| *Neochloris sp.* | 3 | Green | Freshwater |
| *Nitzschia closterium* | 3 | Diatom | Saltwater |
| *Nitzschia palea* | 1 | Diatom | Saltwater |
| *Pavlova gyrans* | 2 | Chrysophyte | Saltwater |
| *Pavlova lutheri* | 5 | Chrysophyte | Saltwater |
| *Phaeodactylum tricornutum* | 15 | Diatom | Saltwater |
| *Platymonas sp.* | 3 | Green | Freshwater |
| *Porphyra yezoensis* | 1 | Macroalga-Red* | Saltwater |
| *Porphyridium cruentum* | 4 | Red | Saltwater |
| *Scenedesmus costatum* | 1 | Green | Freshwater |
| *Scenedesmus subspicatus* | 3 | Green | Freshwater |
| *Stauroneis amphoroides* | 2 | Diatom | Saltwater |
| *Tetraselmis suecica* | 1 | Green | Saltwater |
| *Thalassiosira fluviatilis* | 3 | Diatom | Saltwater |
| *Thalassiosira guillardii* | 1 | Diatom | Saltwater |
| *Thalassiosira pseudonana* | 1 | Diatom | Saltwater |
| **Total** | 122 | | |

[a]The macroalgae with an asterisk were included from Di Landa *et al.* 2009. All other species are from OPP's Ecotoxicity Database of test results submitted during the registration process. Note, if multiple $EC_{50}$ values for a given species were available, then a separate ratio was calculated for each.

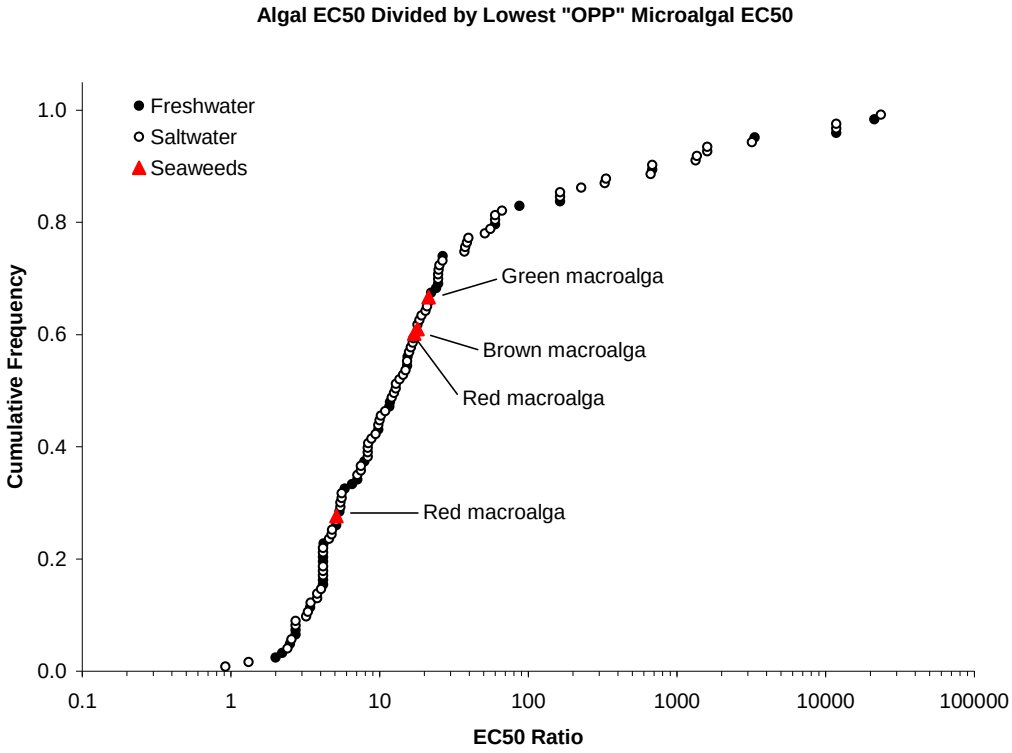


**Figure 2. Cumulative frequency plot of the 122 algal $EC_{50}$ ratios calculated with the most sensitive OPP standard species.**

### 5.1.2. Vascular species

OPP's Ecotoxicity Database has very little data for aquatic vascular species other than those for the freshwater genus *Lemna*. Therefore, to do a similar sensitivity evaluation as was performed for algae, additional data were selected from the published literature within which data for various aquatic vascular plants and at least one species of *Lemna* also were included. Data were restricted in this manner to minimize differences in test conditions between the two $EC_{50}$ values used for each ratio. This should not be considered either an exhaustive search of the literature, or necessarily the optimal way to select data. It is merely one way to begin the process of evaluating the relative sensitivity of *Lemna*. Data were included from seven different publications (Aida *et al*., 2006; Cedergreen *et al*. 2004a,b; Fairchild *et al*. 1998; Forney and Davis 1981; Hanson and Solomon 2004, and Lande *et al*. 2009). *Lemna minor* and *L. gibba* were the species usually represented in OPP data registration packages. In one case (Di Lande *et al*. 2009) data were available for both species of *Lemna*, and the geometric mean of the $EC_{50}$ values were used to establish the $EC_{50}$ ratios[17]. In another case, only data for *L. perpusilla* were

[17] In this instance, by using the *Lemna* geometric mean, a genus-level value is used, while other comparisons are on the species-level. Using the geometric mean is reflective of how OW typically utilizes multiple species in one genus, while OPP would use the lowest species value. This difference will be explored in future analyses.

available (Forney and Davis 1981) and they were used because of the plant's similarity in habitat to the two more commonly used species. However, when data for *L. trisculca* were included in a publication (Cedergreen *et al*. 2004*a*,b), they were treated as a non-standard species for the purpose of this evaluation because it is a submerged species of *Lemna*. Data were available from 11 chemicals and 19 different non-standard aquatic vascular plants, representing 58 separate toxicity tests (**Tables 4** and **5**). Eight of the species were dicots, nine were monocots, and two were aquatic ferns. Exposure durations were between 4 and 14 days for *Lemna* (one test using *L. perpusilla* lasted 28 days) and endpoints were $EC_{50}$ values for some measure of population growth rate (*i.e*., either number of fronds or relative growth rate). Exposure durations for other aquatic vascular plants ranged from 14 to 28 days. Endpoints were $EC_{50}$ values for different measures of individual plant growth—for example, leaf area, wet or dry weight, stem length, or relative growth rate. See **Appendix A** for a list of all of the data used.

Unlike the data for algae, the $EC_{50}$ values for aquatic vascular plants are not consistently greater than those for *Lemna* spp. tests. *Lemna* was the most sensitive species for only one chemical – metribuzin. There was no pattern to the position of the relative sensitivity among the non-standard species whether compared by either species or chemical tested (**Figure 3**). Of the $EC_{50}$ values that were more sensitive compared to those for *Lemna,* the majority of the $EC_{50}$ values were within a factor of 10 of the *Lemna* $EC_{50}$ value. In fact, only the $EC_{50}$ for the floating fern *Salvinia natans* tested using bensulfuron methyl was outside this factor of 10. A 10-fold extrapolation factor applied to *Lemna* data has been suggested by Rentz and Hanson (2009) to be more protective of other macrophytes. A factor of 10 also is used by the European Union and Canada in their lower-tiered assessments; however, this factor is applied to the lowest value whether plant or animal (these methods, along with methods employed by other countries are briefly summarized in Section 3.5). The use of the factor of 10 is not being advocated in this document, but its use will be considered as an option after further data analysis. As with the microalgal data, the *Lemna* analysis should be interpreted with caution given the limited number of plant groups represented. Most notably, data for near coastal marine species are particularly sparse, (*e.g*., saltmarsh species, seagrasses and mangroves).

The concern about the representativeness of *Lemna* effects data for other groups of aquatic vascular plants was a common issue at a recent European workshop held in the Netherlands in 2008 (Maltby *et al*. 2010). A standardized test procedure using species of *Myriophyllum* and synthetic sediment is being evaluated to fill the above needs for European risk assessments (Maltby *et al*. 2010). *Myriophyllum* was selected because it is a dicot (*Lemna* is a monocot) and is a rooted aquatic vascular plant. A dicot is desirable in part because many herbicides target broadleaf "weeds" (dicots). The data analyses suggest that if resources are limited, then the most significant data to add to a minimal data set would be for additional aquatic vascular plants. There is more uncertainty associated with the sensitivity of this portion of the plant community than for the microalgal portion.

**Table 4. Chemicals selected from the open scientific literature for which ratios of $EC_{50}$ values for vascular plants were calculated[a].**

| Chemical | N |
|---|---|
| Alachlor | 4 |
| Atrazine | 4 |
| Bensulfuron methyl | 2 |
| Chlorodifluoroacetic acid | 2 |
| Dichloroacetic acid | 2 |
| Metolachlor | 4 |
| Metribuzin | 7 |
| Metsulfuron-methyl | 11 |
| Monochloroacetic acid | 2 |
| Terbuthylazine | 13 |
| Trichloroacetic acid | 2 |
| Trifluoroacetic acid | 2 |
| Irgarol | 3 |
| Total | 58 |

[a]The denominator of the ratio was the $EC_{50}$ from the available *Lemna* species. N = the total number of available $EC_{50}$ values for non-OPP species for each chemical. Note, if multiple $EC_{50}$ values for a given species were available, then a separate ratio was calculated for each.

**Table 5. List of the non-standard vascular aquatic plant species used to calculate the $EC_{50}$ ratios with *Lemna*[a].**

| Species | Group | N |
|---|---|---|
| *Batrachium trichophyllum* | dicot | 1 |
| *Berula erecta* | dicot | 1 |
| *Caboma caroliniana* | dicot | 1 |
| *Ceratophyllum demersum* | dicot | 6 |
| *Ceratophyllum submersum* | dicot | 3 |
| *Myriophyllum heterophyllum* | dicot | 4 |
| *Myriophyllum sibiricum* | dicot | 2 |
| *Myriophyllum spicatum* | dicot | 8 |
| *Callitriche platycarpa* | monocot | 2 |
| *Elodea canadensis* | monocot | 9 |
| *Lemna trisulca* | monocot | 2 |
| *Najas sp.* | monocot | 4 |
| *Potamogeton crispus* | monocot | 3 |
| *Potamogeton pectinatus* | monocot | 1 |
| *Ruppia maritima*[b] | monocot | 1 |
| *Spirodela polyrhiza* | monocot | 4 |
| *Zostera marina*[b] | monocot | 1 |
| *Azolla japonica* | fern | 1 |
| *Salvinia natans* | fern | 1 |
| **Total** | | 58 |

[a]N = the total number of available $EC_{50}$ values for each species. Note, if multiple $EC_{50}$ values for a given species were available, then a separate ratio was calculated for each.

[b]Marine macrophytes

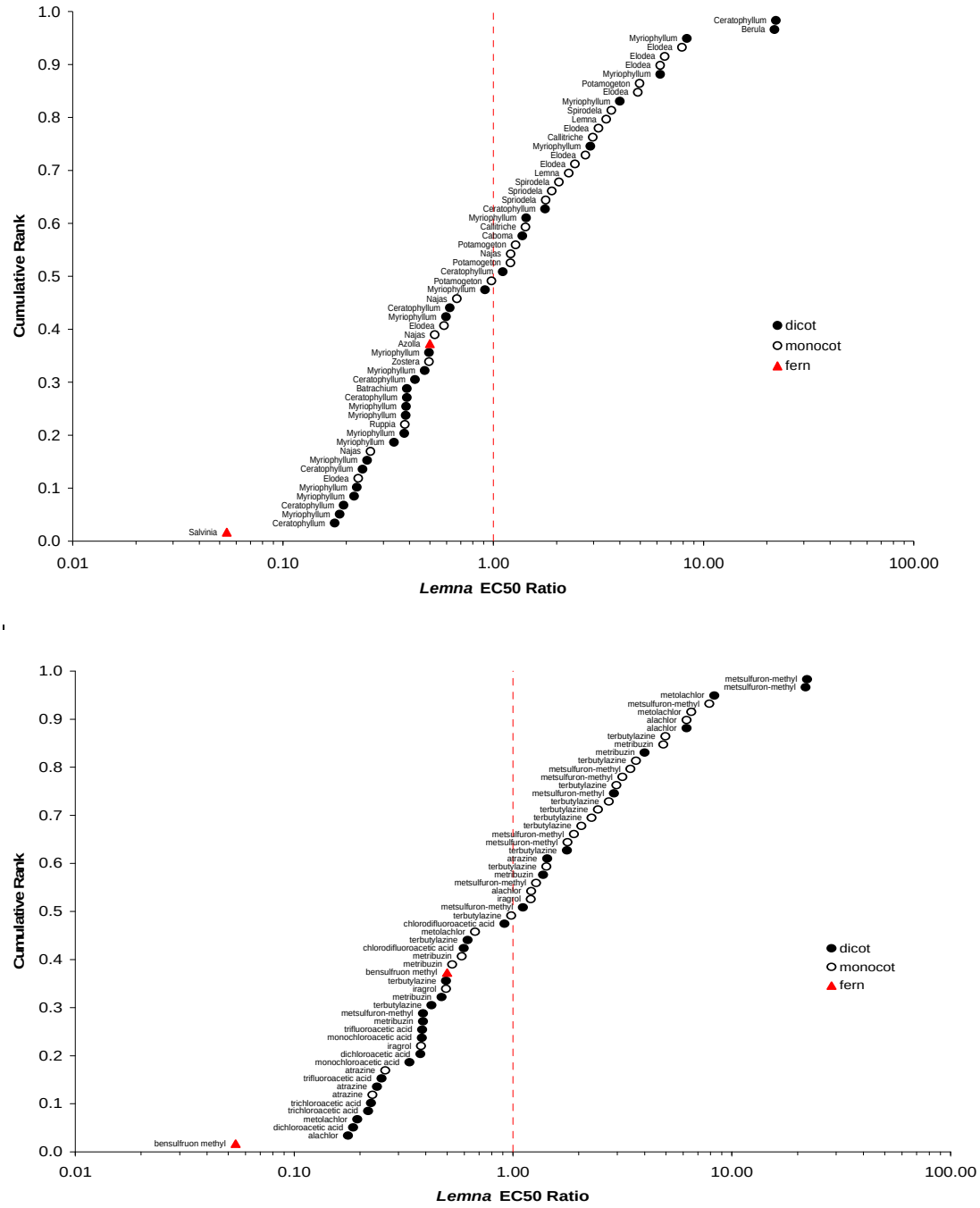


**Figure 3. Cumulative frequency plot of the 58 aquatic vascular plant $EC_{50}$ ratios with *Lemna*. Both plots have same data; the top is labeled by species and the bottom is labeled by chemical.**

### 5.2. Species sensitivity distributions

For OPP's risk assessments, data for non-vascular and vascular plants are used separately. This is one reason why the analyses in the last section were done with the data

segregated into these two groups. Another approach is to combine all data for both groups into one assessment. This is what was done for the species sensitivity distribution used for the earlier mentioned acetochlor, metolachlor and atrazine. These SSD approaches separated the data into freshwater and saltwater; acetochlor and metolachlor only used freshwater data and the draft atrazine document created separate distributions for each medium. The analyses described below combines all data from all taxonomic groups and both freshwater and saltwater.

One of the underlying issues related to determining an appropriate toxicity benchmark (or criterion) for aquatic plants is that there is not a consensus (or, unlike aquatic animals, even a historical precedence) for what constitutes a minimum data set which would represent the range of sensitivities for a given plant community. However, a number of existing data sets can be found in which numerous data points exist for a variety of species representing many niches in the aquatic plant community. For example there are 44 species for which diuron $EC_{50}$ values exist, 38 for irgarol, 31 for pentachlorophenol, 25 for atrazine, 21 for metolachlor and 13 for diquat. Each of these data sets contains a variety of both non-vascular and vascular plant data, and it is reasonable to expect that these data sets are equal to or greater than some as yet undetermined "representative minimum data set". Each of these data sets also contains data for all of the OPP "standard species".

Not only is there no consensus on what constitutes a minimum data set of species, there is also no consensus on what the appropriate durations of exposure are or what the most appropriate measurement endpoints are —with the possible exception of microalgae. The few times that a large data set has been used for setting plant values (*e.g.*, acetochlor, metolachlor, atrazine) a variety of durations and measurement endpoints have been used. This is largely because most of the plant data used in these SSDs came from studies whose goals were not regulatory in nature—data collected for a variety of reasons, by its nature would have a variety of durations and endpoints. A variety of endpoints are included in the sample SSD data sets. The data are listed in **Appendix B.**

Both freshwater and saltwater data for aquatic plants were downloaded from the USEPA's ECOTOX database[18] in May 2010 for four of the herbicides and pentachlorophenol (a wood preservative). The data for atrazine came from the June 2009 draft water quality criteria document, supplemented with data from the OPP Ecotoxicity Database. All data were used "as is" (*i.e.*, original references were not checked). These compounds were selected because a reasonably large number of $EC_{50}$ values were available ($LC_{50}$ and $IC_{50}$ values also were included). It also should be noted that many of these plotted values are based on unmeasured (nominal) exposure concentrations. For all compounds, the geometric mean was calculated for species with more than one $EC_{50}$ for a compound. SSDs for all six compounds are plotted in **Figure 4.** To demonstrate how a benchmark derived using only the OPP standard test species compares to a benchmark value derived using a larger, more diverse data set, two separate species sensitivity distributions were plotted for each chemical. One SSD included all of the data and the

[18] http://cfpub.epa.gov/ecotox

other included just the data from the OPP standard species. **Equation 1**[19] was fit to each of the "full" and "partial" data sets using Microsoft Excel's solver routine. $HC_5$ values were calculated using the resulting fitted equations, as well as the method OW typically uses to develop final acute values for animals (USEPA 1985)[20]. These data are shown in **Table 6.**

$$Cummulative_{Frequency} = \frac{1}{1+\left(\frac{C}{X_m}\right)^S} \qquad \textbf{Equation 1}$$

Where:

$C$ = $EC_{50}$ value,
$X_m$ = median $EC_{50}$ value, and
$S$ = shape factor (slope)

**Table 6. Comparison of calculated plants values using three different approaches. All values are µg/L.**

| | | Calculated $HC_5$[b] | | FAV 5th percentile[d] | | OPP data[e] | |
|---|---|---|---|---|---|---|---|
| **Compound** | **N[a]** | **Full data set** | **Partial Data[c]** | **Full data set** | **Partial Data[c]** | **Lowest $EC_{50}$** | **Species** |
| Atrazine | 25 | 10.37 | 9.69 | 13.79 | 50.52 | 50.5 | *Lemna gibba* |
| Diquat | 13 | 0.647 | 1.154 | 0.491 | 4.07 | 5.1 | *Lemna minor* |
| Diuron | 44 | 1.68 | 4.04 | 2.84 | 13.89 | 8.0 | *Pseudokirchneriella subcapitata* |
| Irgarol | 38 | 0.097 | 0.032 | 0.107 | 0.415 | 0.1 | *Navicula pelliculosa* |
| Metolachlor | 21 | 12.47 | 6.02 | 38.88 | 29.69 | 34.2 | *Pseudokirchneriella subcapitata* |
| Pentachlorophenol | 31 | 15.26 | 8.30 | 39.69 | 49.11 | 35.3 | *Skeletonema costatum* |

[a] Number of species mean plant values
[b] Calculated using the fitted Equation 1to all of the SSD data
[c] Only the data for the OPP standard species are included
[d] Calculated using the four most sensitive values—the Final Acute Value method from USEPA 1985
[e] Using only the data for the OPP standard species. If lower acceptable test values are available to OPP, those species would be used for deriving an OPP benchmark. For instance, the actual benchmark for atrazine is 1µg/L.

[19] There are a variety of possible sigmoid equations that could be used. This one is shown only as one example for the purpose of demonstration.
[20] OW's 1985 guidelines' procedure for FAV calculation is essentially a regression using the four most sensitive genus mean values (species means used for FVP). The calculated value represents the cumulative probability of 0.05 of all the test values. Note that in other countries (as well as for the Minnesota calculations for acetochlor and metolachlor) SSDs are used by fitting a sigmoid curve to the data and calculating a percentile effect concentration (*e.g.*, $EC_{05}$ or $EC_{20}$).

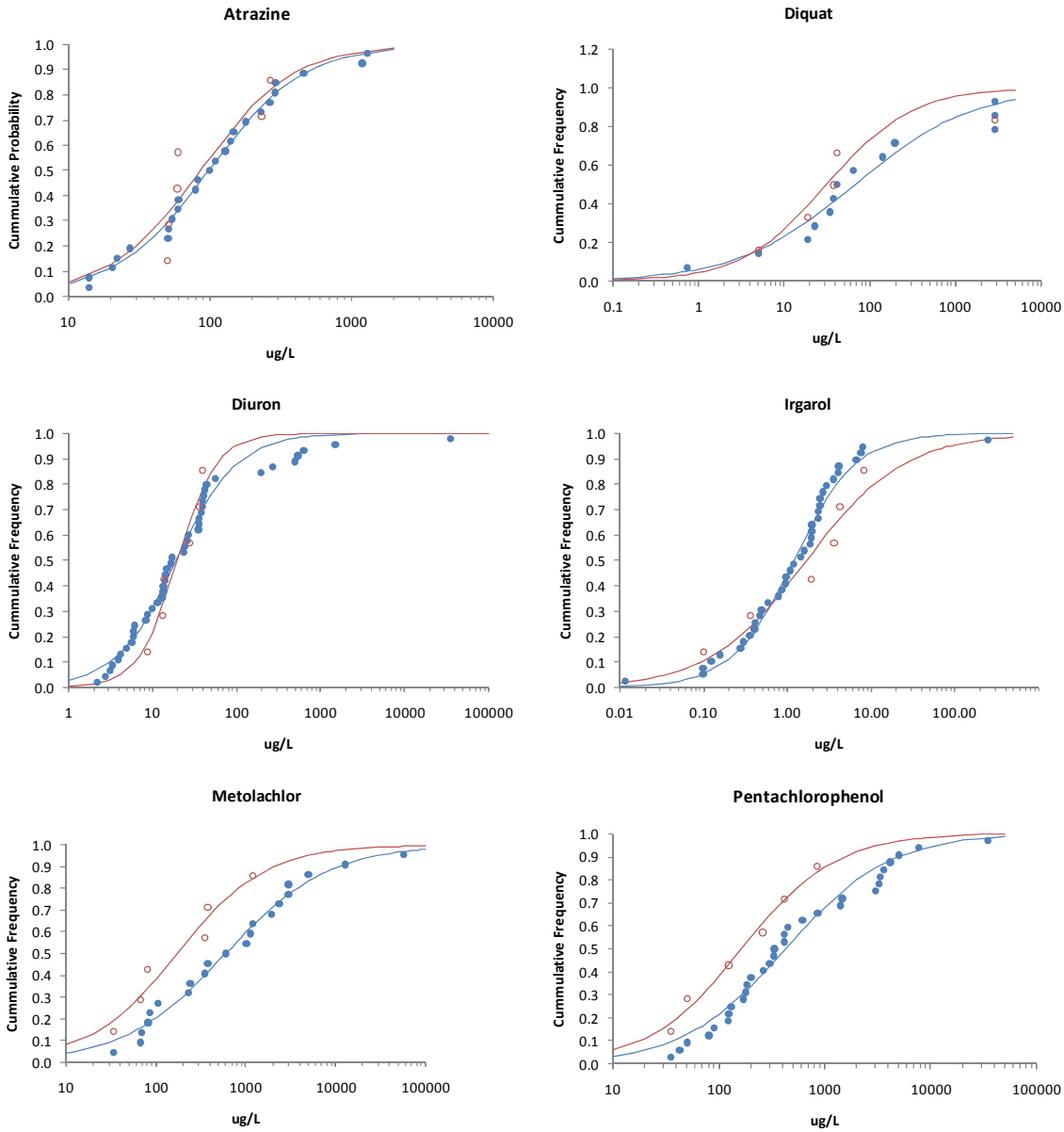


**Figure 4. Species sensitivity distributions for aquatic plants using species mean $EC_{50}$ values. See Appendix B for data. Closed circles represent all of the available data. Open circles are the subsets of the data containing only the data for the OPP standard species.**

The information in **Table 6** demonstrates that a different benchmark value is derived if more data are available and if different approaches are used. It should be noted that for most of the example chemicals, the lowest OPP value falls nearly at the $HC_{10}$ of the distribution. The $HC_5$ approach with the full data set are the only derived values that use all of the available data, and is often the approach preferred when plant data are evaluated[21]. While the fitting of a curve to the full data set will probably result in the best estimate of the "true" benchmark for a given compound, the method used above (*i.e.*, **Equation 1**) should not be taken as the best. Likewise, the selection of the $HC_5$ as the point of interest is presented here only as one example. In addition, these data suggest that an factor may be needed in order to extrapolate from the minimal data set (e.g., data from only the OPP standard species).

## 6. Potential approach for deriving plant ALSV

The amount of aquatic plant toxicity data available varies among pesticides. As indicated above, toxicity data are required for pesticides; however, data are often available in the scientific literature, particularly for herbicides (*e.g.*, atrazine, metolachlor). In cases where plants are expected to be more sensitive than animals (*i.e.*, for herbicides), it may be necessary to derive a plant ALSV with which to compare against the animal ALSV. The plant ALSV is based on phytotoxicity and its calculation excludes animal toxicity data which are used for the animal ALSV calculation alone.

Different approaches for deriving this value may be used, depending upon availability of data. The plant ALSV may be represented by the lowest single toxicity value for aquatic plants. In cases where there is evidence to suggest that the available toxicity data are not representative of the most sensitive plant species which are expected to be impacted, extrapolation factors may be applied to available data to derive the plant ALSV. In deciding whether to use an extrapolation factor, the chemical's mode of action on aquatic plants and whether the available test species are likely to be impacted should be considered. If a chemical has a large data set (*e.g.,* atrazine, metolachlor), it may be possible to use a SSD to derive a plant ALSV. Additionally, as discussed in the animal white paper, predictive tools for estimating toxicity values may be available which could allow further development of plant SSDs when combined with existing data. As discussed in the tools white paper though, there are a limited number of predictive tools at this time for estimating the toxicity of chemicals to aquatic plants. Where such tools are available, they may only predict for a limited number of aquatic plants, *e.g.*, freshwater algae alone. However, efforts are underway to enhance the predictive capabilities of [quantitative] structure activity relationship ([Q]SAR) models through inclusion of plant toxicity data in model training sets. Use of other predictive methods such as analogs and read-across (discussed in the tools white paper) may provide the user with an understanding of the extent existing data for the chemical in question are reflective of plant sensitivity for similarly structured chemicals. This information could provide a rationale for using existing data alone, reliance on an extrapolation factor, or

[21] By "approach preferred" we mean that fitting a sigmoid curve to the full data set is often the preferred approach. Different equations may be used, as well as different percentiles (*e.g.*, $HC_{20}$).

possibly using these analog/read-across and/or [Q]SAR estimates as a means of populating SSDs which would in turn be used for developing a plant ALSV.

It is expected that once the methods described here are reviewed, the approach for deriving plant ALSVs will be revised to be more specific and to incorporate the methods determined to have the greatest utility. In order to refine this approach, it will be necessary to define the endpoints used in deriving a plant ALSV. This includes considering the desired duration of exposure, level of effect (*e.g.*, $EC_{50}$) and measurement endpoint (*e.g.*, growth rate) that are used to define the toxicity data for the ALSV. Also, it will be necessary to define the assessment endpoint, *i.e.*, survival, growth or reproduction, which the ALSV is intended to represent. Finally, it will be necessary to define the community effect level (*e.g.*, $HC_x$) if an SSD approach is used.

In addition to the above, an approach for determining appropriate EFs for when different amounts of plant data are available can also be undertaken. The minimum amount of data considered will be the data for the OPP "standard species". This is because any new or reregistered pesticide should have a minimum of five data points (four microalgal points and one for *Lemna* sp.). The approach will consist of finding as many data sets as possible for which a large number of $EC_{50}$ values already exist. This will include non-pesticide data in order to maximize the number of chemicals represented. The non-pesticide data sets also will need to contain data that would meet the OPP data requirements for aquatic plants. With enough additional data sets like the six presented as SSDs in this document, EFs could be developed that could be used to account for when only the standard set of OPP data are available. When more data than the standard OPP data set are available, one approach would be to return to the data sets and start adding some of the non-OPP standard species data to the OPP standard set and observing how the "partial" SSD equation changes. It will get closer to matching the equation for the "full" plot as more data are added. This can be done for all possible combinations of adding data in groups of 1, 2, 3, *etc*. Using the "full" SSD to represent the best benchmark, recommendations could be made on how the EF should be altered as the number of data points increases. Based on the plots presented above in **Figures 2 and 3**, the analysis could concentrate on adding back data for aquatic vascular plants. This may also shed some light on how many and/or what species reduce the difference between the full and partial curves the quickest. In relation to the derivation of SSDs and EFs, future analyses will explore the different sources of uncertainty in plant data, including those described above.

## 7. Conclusions

This white paper presents several methods used to characterize the effects of stressors on aquatic plant communities. These methods include use of the most sensitive empirical toxicity data, extrapolation factors, and sensitivity distributions. This paper also explores uncertainties associated with aquatic plant toxicity data, including the sensitivities of typical test species relative to other aquatic plants for which test species are surrogates. Based upon methodologies used by other countries and by U.S. regulatory agencies, and the analyses of relative sensitivities of the standard OPP test species, plant community

benchmark values can logically be derived. The amount of data available for plant species should be considered when calculating a plant ALSV. For pesticides, data are routinely available for the standard five OPP test species. It may be possible to use the lowest of the available data to represent the plant ALSV (either alone or with extrapolation factors). When additional data are available in addition to the standard OPP test species, SSDs have proven effective for deriving toxicity values which are considered reflective of the sensitivity of more vulnerable aquatic plant species. Possible approaches for deriving plant ALSVs are broadly described which are similar to those discussed in the aquatic animal white paper. These approaches are intended to account for uncertainties and to make the best use of available data.

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## Appendix A. Vascular plant (VP) data used in creating ratios comparing *Lemna* to other VPs

| Species | Chemical | Duration (d) | Endpoint | $EC_{50}$ (µg/L) | Reference |
|---|---|---|---|---|---|
| *Ceratophyllum demersum* | alachlor | 14 | increase in wet wt | 85 | Farichild et al. 1998 |
| *Elodea canadensis* | alachlor | 14 | increase in wet wt | > 3000 | Farichild et al. 1998 |
| *Lemna minor* | alachlor | 4 | # fronds | 482 | Farichild et al. 1998 |
| *Myriophyllum heterophyllum* | alachlor | 14 | increase in wet wt | > 3000 | Farichild et al. 1998 |
| *Najas* sp. | alachlor | 14 | increase in wet wt | 584 | Farichild et al. 1998 |
| *Ceratophyllum demersum* | atrazine | 14 | increase in wet wt | 22 | Farichild et al. 1998 |
| *Elodea canadensis* | atrazine | 14 | increase in wet wt | 21 | Farichild et al. 1998 |
| *Lemna minor* | atrazine | 4 | # fronds | 92 | Farichild et al. 1998 |
| *Myriophyllum heterophyllum* | atrazine | 14 | increase in wet wt | 132 | Farichild et al. 1998 |
| *Najas* sp. | atrazine | 14 | increase in wet wt | 24 | Farichild et al. 1998 |
| *Azolla japonica* | bensulfruon methyl | 12 | relative growth rate | 5 | Aida et al. 2006 |
| *Lemna minor* | bensulfruon methyl | 12 | relative growth rate | 10 | Aida et al. 2006 |
| *Salvinia natans* | bensulfruon methyl | 12 | relative growth rate | 0.54 | Aida et al. 2006 |
| *Lemna gibba* | chlorodifluoroacetic acid | 7 | frond # | 176.4 | Hanson & Solomon 2004a |
| *Myriophyllum sibiricum* | chlorodifluoroacetic acid | 14 | root length | 161.4 | Hanson & Solomon 2004a |
| *Myriophyllum spicatum* | chlorodifluoroacetic acid | 14 | root length | 105.3 | Hanson & Solomon 2004a |
| *Lemna gibba* | dichloroacetic acid | 7 | frond # | 199.2 | Hanson & Solomon 2004a |
| *Myriophyllum sibiricum* | dichloroacetic acid | 14 | root length | 75.3 | Hanson & Solomon 2004a |
| *Myriophyllum spicatum* | dichloroacetic acid | 14 | root length | 37.1 | Hanson & Solomon 2004a |
| *Potamogeton pectinatus* | iragrol | | | 6.10 | Di Lande et al. 2009 |
| *Ruppia maritima* | iragrol | | | 1.92 | Di Lande et al. 2009 |

| | | | | | |
|---|---|---|---|---|---|
| *Zostera marina* | iragrol | | | 2.50 | Di Lande et al. 2009 |
| *Ceratophyllum demersum* | metolachlor | 14 | increase in wet wt | 70 | Farichild et al. 1998 |
| *Elodea canadensis* | metolachlor | 14 | increase in wet wt | 2355 | Farichild et al. 1998 |
| *Lemna minor* | metolachlor | 4 | # fronds | 360 | Farichild et al. 1998 |
| *Myriophyllum heterophyllum* | metolachlor | 14 | increase in wet wt | > 3000 | Farichild et al. 1998 |
| *Najas* sp. | metolachlor | 14 | increase in wet wt | 242 | Farichild et al. 1998 |
| *Caboma caroliniana* | metribuzin | 21 | stem length | 22 | Forney & Davis 1981 |
| *Ceratophyllum demersum* | metribuzin | 14 | increase in wet wt | 14 | Farichild et al. 1998 |
| *Elodea canadensis* | metribuzin | 14 | increase in wet wt | 21 | Farichild et al. 1998 |
| *Elodea canadensis* | metribuzin | 21 | stem length | 78 | Forney & Davis 1981 |
| *Lemna minor* | metribuzin | 4 | # fronds | 36 | Farichild et al. 1998 |
| *Lemna perpusilla* | metribuzin | 28 | new plants | 16 | Forney & Davis 1981 |
| *Myriophyllum heterophyllum* | metribuzin | 14 | increase in wet wt | 17 | Farichild et al. 1998 |
| *Myriophyllum spicatum* | metribuzin | 28 | stem length | 64 | Forney & Davis 1981 |
| *Najas* sp. | metribuzin | 14 | increase in wet wt | 19 | Farichild et al. 1998 |
| *Batrachium trichophyllum* | metsulfuron-methyl | 14 | specific leaf area | 0.07 | Cedergreen et al. 2004 a |
| *Berula erecta* | metsulfuron-methyl | 14 | specific leaf area | 3.92 | Cedergreen et al. 2004 a |
| *Ceratophyllum demersum* | metsulfuron-methyl | 14 | specific leaf area | 0.2 | Cedergreen et al. 2004 a |
| *Ceratophyllum submersum* | metsulfuron-methyl | 14 | specific leaf area | 2.21 | Cedergreen et al. 2004 a |
| *Elodea canadensis* | metsulfuron-methyl | 14 | specific leaf area | 0.57 | Cedergreen et al. 2004 a |
| *Elodea canadensis* | metsulfuron-methyl | 14 | specific leaf area | 0.79 | Cedergreen et al. 2004 a |
| *Lemna minor* | metsulfuron-methyl | 14 | relative growth rate | 0.8 | Cedergreen et al. 2004 a |
| *Lemna minor* | metsulfuron-methyl | 14 | relative growth rate | 1.13 | Cedergreen et al. 2004 a |
| *Lemna minor* | metsulfuron-methyl | 14 | specific leaf area | 0.18 | Cedergreen et al. 2004 a |

| | | | | | |
|---|---|---|---|---|---|
| *Lemna minor* | metsulfuron-methyl | 14 | specific leaf area | 0.1 | Cedergreen et al. 2004 a |
| *Lemna trisulca* | metsulfuron-methyl | 14 | specific leaf area | 0.62 | Cedergreen et al. 2004 a |
| *Myriophyllum spicatum* | metsulfuron-methyl | 14 | specific leaf area | 0.29 | Cedergreen et al. 2004 a |
| *Potamogeton crispus* | metsulfuron-methyl | 14 | specific leaf area | 0.23 | Cedergreen et al. 2004 a |
| *Spriodela polyrhiza* | metsulfuron-methyl | 14 | specific leaf area | 0.32 | Cedergreen et al. 2004 a |
| *Spriodela polyrhiza* | metsulfuron-methyl | 14 | specific leaf area | 0.19 | Cedergreen et al. 2004 a |
| *Lemna gibba* | monochloroacetic acid | 7 | frond # | 17.2 | Hanson & Solomon 2004a |
| *Myriophyllum sibiricum* | monochloroacetic acid | 14 | root length | 5.8 | Hanson & Solomon 2004a |
| *Myriophyllum spicatum* | monochloroacetic acid | 14 | root length | 6.6 | Hanson & Solomon 2004a |
| *Callitriche platycarpa* | terbutylazine | 14 | dry weight | 158 | Cedergreen et al. 2004b |
| *Callitriche platycarpa* | terbutylazine | 14 | dry weight | 119 | Cedergreen et al. 2004b |
| *Ceratophyllum demersum* | terbutylazine | 14 | dry weight | 196 | Cedergreen et al. 2004b |
| *Ceratophyllum submersum* | terbutylazine | 14 | dry weight | 17 | Cedergreen et al. 2004b |
| *Ceratophyllum submersum* | terbutylazine | 14 | dry weight | 69 | Cedergreen et al. 2004b |
| *Elodea canadensis* | terbutylazine | 14 | dry weight | 98 | Cedergreen et al. 2004b |
| *Elodea canadensis* | terbutylazine | 14 | dry weight | 305 | Cedergreen et al. 2004b |
| *Lemna minor* | terbutylazine | 14 | dry weight | 40 | Cedergreen et al. 2004b |
| *Lemna minor* | terbutylazine | 14 | dry weight | 111 | Cedergreen et al. 2004b |
| *Lemna trisulca* | terbutylazine | 14 | dry weight | 254 | Cedergreen et al. 2004b |
| *Myriophyllum spicatum* | terbutylazine | 14 | dry weight | 55 | Cedergreen et al. 2004b |
| *Potamogeton crispus* | terbutylazine | 14 | dry weight | 109 | Cedergreen et al. 2004b |
| *Potamogeton crispus* | terbutylazine | 14 | dry weight | 199 | Cedergreen et al. 2004b |
| *Spirodela polyrhiza* | terbutylazine | 14 | dry weight | 228 | Cedergreen et al. 2004b |
| *Spirodela polyrhiza* | terbutylazine | 14 | dry weight | 146 | Cedergreen et al. 2004b |

| | | | | | |
|---|---|---|---|---|---|
| *Lemna gibba* | trichloroacetic acid | 7 | frond # | 254.1 | Hanson & Solomon 2004a |
| *Myriophyllum sibiricum* | trichloroacetic acid | 14 | root length | 55.4 | Hanson & Solomon 2004a |
| *Myriophyllum spicatum* | trichloroacetic acid | 14 | root length | 57.1 | Hanson & Solomon 2004a |
| *Lemna gibba* | trifluoroacetic acid | 7 | frond # | 884 | Hanson & Solomon 2004a |
| *Myriophyllum sibiricum* | trifluoroacetic acid | 14 | root length | 340.7 | Hanson & Solomon 2004a |
| *Myriophyllum spicatum* | trifluoroacetic acid | 14 | root length | 222.1 | Hanson & Solomon 2004a |

## Appendix B. Data for Species Sensitivity Distribution plots

### Atrazine

| Species | Common name | Medium | Species group | Duration (d) | Endpoint | $EC_{50}/IC_{50}$ or $LC_{50}$[a,b] (µg/L) | SMPV (µg/L) |
|---|---|---|---|---|---|---|---|
| *Anabaena flos-aquae* | Cyanobacterium | FW | Non-vascular | 5 | | 230[a] | 230 |
| *Chlamydomonas reinhardtii* | Green alga | FW | Non-vascular | 4 | cell number | 51[b] | 27.32 |
| *Chlamydomonas reinhardtii* | Green alga | FW | Non-vascular | 4 | cell number | 51[b] | |
| *Chlamydomonas reinhardtii* | Green alga | FW | Non-vascular | 7 | cell number | 21[b] | |
| *Chlamydomonas reinhardtii* | Green alga | FW | Non-vascular | 10 | cell number | 10.2[b] | |
| *Chlorella saccharophila* | Green alga | FW | Non-vascular | 4 | cell number | 1,300[b] | 1,300 |
| *Chlorella* sp. | Green alga | SW | Non-vascular | 3 | growth | 140[b] | 140 |
| *Dunaliella tertiolecta* | Green alga | SW | Non-vascular | 5 | | 180[a] | 180 |
| *Elodea canadensis* | Elodea | FW | Vascular | 10 | biomass | 1200[b] | 1,200 |
| *Isochrysis galbana* | Golden/brown alga | SW | Non-vascular | 5 | | 22[a] | 22 |
| *Lemna gibba* | Duckweed | FW | Vascular | 7 | frond production | 180[b] | 50.54 |
| *Lemna gibba* | Duckweed | FW | Vascular | 14 | frond number | 37[b] | |
| *Lemna gibba* | Duckweed | FW | Vascular | 14 | frond biomass | 45[b] | |
| *Lemna gibba* | Duckweed | FW | Vascular | 14 | frond biomass | 22[b] | |
| *Lemna gibba* | Duckweed | FW | Vascular | 14 | frond number | 50[b] | |
| *Lemna minor* | Duckweed | FW | Vascular | 14 | biomass | 8700[b,*] | |
| *Lemna minor* | Duckweed | FW | Vascular | 10 | frond number | 56[b] | 59.28 |
| *Lemna minor* | Duckweed | FW | Vascular | 10 | fresh weight | 60[b] | |

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|---|---|---|---|---|---|---|---|
| *Lemna minor* | Duckweed | FW | Vascular | 10 | chlorophyll | 62 [b] | |
| *Microcystis aeruginosa* | Cyanobacterium | FW | Non-vascular | 5 | | 129[a] | 129 |
| *Myriophyllum spicatum* | Eurasian water milfoil | SW | Vascular | 28 | photosynthesis | 117 [b] | 54.08 |
| *Myriophyllum spicatum* | Eurasian water milfoil | SW | Vascular | 35 | biomass | 25 [b] | |
| *Navicula incerta* | Diatom | SW | Non-vascular | 3 | | 460[a] | 460 |
| *Navicula pelliculosa* | Diatom | FW | Non-vascular | 5 | | 60[a] | 60 |
| *Neochloris* sp. | Green alga | SW | Non-vascular | 3 | growth | 82 [b] | 82 |
| *Nitzschia closterium* | Diatom | SW | Non-vascular | 3 | | 290[a] | 290 |
| *Pavlova* sp. | Golden/brown alga | SW | Non-vascular | 4 | growth | 147 [b] | 147 |
| *Platymonas* sp. | Green alga | SW | Non-vascular | 3 | | 100 [b] | 100 |
| *Porphyridium cruentum* | Red alga | SW | Non-vascular | 3 | growth | 79 [b] | 79 |
| *Potamogeton perfoliatus* | Redheadgrass pondweed | SW | Vascular | 28 | photosynthesis | 55 [b] | 20.49 |
| *Potamogeton perfoliatus* | Redheadgrass pondweed | SW | Vascular | 35 | final biomass | 30 [b] | |
| *Pseudanabaena geleata* | Cyanobacterium | FW | Non-vascular | 4 | cell number | 14 [b] | 14 |
| *Pseudokirchneriella subcapitata* | Green alga | FW | Non-vascular | 4 | cell number | 4 [b] | 51.04 |
| *Pseudokirchneriella subcapitata* | Green alga | FW | Non-vascular | 4 | phaeophytin-a | 20 [b] | |
| *Pseudokirchneriella subcapitata* | Green alga | FW | Non-vascular | 4 | chlorophyll-a | 150 [b] | |
| *Pseudokirchneriella subcapitata* | Green alga | FW | Non-vascular | 4 | cell number | 128.2 [b] | |
| *Pseudokirchneriella subcapitata* | Green alga | FW | Non-vascular | 4 | cell number | 130 [b] | |
| *Pseudokirchneriella subcapitata* | Green alga | FW | Non-vascular | 5 | | 55 [b] | |
| *Pseudokirchneriella subcapitata* | Green alga | FW | Non-vascular | 4 | growth | 82 [b] | |
| *Scenedesmus acutus* | Green alga | FW | Non-vascular | 4 | cell number | 14 [b] | 14 |
| *Skeletonema costatum* | Diatom | SW | Non-vascular | 2 | growth | 265 [b] | 265 |
| *Thalassiosira fluviatilis* | Diatom | SW | Non-vascular | 3 | | 110[a] | 110 |

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|---|---|---|---|---|---|---|---|
| *Zostera marina* | Eelgrass | SW | Vascular | 21 | survival | 540 [b] | 291.6 |
| *Zostera marina* | Eelgrass | SW | Vascular | 21 | survival | 100 [b] | |
| *Zostera marina* | Eelgrass | SW | Vascular | 21 | survival | 365 [b] | |
| *Zostera marina* | Eelgrass | SW | Vascular | 21 | survival | 367 [b] | |

*Not used in SMPV
[a]$EC_{50}/IC_{50}/LC_{50}$ data from OPP database
[b]$EC_{50}/IC_{50}/LC_{50}$ data from WQC draft (June 23, 2009)

## Diquat

| Species | Common name | Medium | Species Group | Duration (d) | Endpoint | $EC_{50}/IC_{50}$ [a] (µg/L) | SMPV (µg/L) |
|---|---|---|---|---|---|---|---|
| *Anabaena flos aquae* | Cyanobacterium | FW | Non-Vascular | 3 | growth rate | 42 | 42 |
| *Anacystis aeruginosa* | Cyanobacterium | FW | Non-Vascular | 3 | growth rate | 65 | 65 |
| *Chlorella vulgaris* | Green algae | FW | Non-Vascular | 3 | growth rate | 2940 | 2940 |
| *Cryptomonas ozolini* | Cryptomonad | FW | Non-Vascular | 3 | population change, general | 35 | 35 |
| *Euglena gracilis* | Flagellate euglenoid | FW | Non-Vascular | 3 | growth rate | 2940 | 2940 |
| *Lemna minor* | Duckweed | FW | Vascular | 7 | growth rate | 1.5 | 5.08 |
| *Lemna minor* | Duckweed | FW | Vascular | 7 | growth rate | 2.7 | |
| *Lemna minor* | Duckweed | FW | Vascular | 7 | growth rate | 3.1 | |
| *Lemna minor* | Duckweed | FW | Vascular | 7 | relative growth rate | 15 | |
| *Lemna minor* | Duckweed | FW | Vascular | 4 | population change, general | 18 | |
| *Lyngbya* sp. | Cyanobacterium | FW | Non-Vascular | 3 | population growth rate | 145 | 145 |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | area | 56.2 | 200.43 |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | number of root | 57 | |

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|---|---|---|---|---|---|---|---|
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | weight | 78.2 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | length | 79.7 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | length | 105.7 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | area | 127.7 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | number of root | 155.1 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | weight | 184 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | abundance | 271.3 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | length | 346.2 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | abundance | 365.7 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | length | 403.8 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | abundance | 982.8 | |
| *Myriophyllum sibiricum* | Water milfoil | FW | Vascular | 14 | length | 1610.9 | |
| *Navicula* sp. | Diatom | FW | Non-Vascular | 3 | growth rate | 19 | 19 |
| *Ochromonas danica* | Diatom | FW | Non-Vascular | 3 | population change, general | 23 | 23 |
| *Pseudokirchneriella subcapitata* | Green algae | FW | Non-Vascular | 4 | abundance | 4.9 | 37.91 |
| *Pseudokirchneriella subcapitata* | Green algae | FW | Non-Vascular | 4 | abundance | 34.2 | |
| *Pseudokirchneriella subcapitata* | Green algae | FW | Non-Vascular | 3 | growth rate | 73 | |
| *Pseudokirchneriella subcapitata* | Green algae | FW | Non-Vascular | 3 | population growth rate | 80 | |
| *Pseudokirchneriella subcapitata* | Green algae | FW | Non-Vascular | 4 | population change, general | 80 | |
| *Skeletonema costatum* | Diatom | FW | Non-Vascular | 3 | growth rate | 2940 | 2940 |
| *Spirodela punctata* | Large duckweed | FW | Vascular | 14 | abundance | 0.75 | 0.75 |

[a]Data from ECOTOX database

## Diuron

| Species | Common Name | Species Group | Medium | Duration (d) | Endpoint | $EC_{50}/IC_{50}/LC_{50}$[a] (µg/L) | SMPV (µg/L) |
|---|---|---|---|---|---|---|---|
| *Anabaena doliolum* | Cyanobacterium | Non-vascular | FW | 12 | population growth rate | 1000 | 632.46 |
| *Anabaena doliolum* | Cyanobacterium | Non-vascular | FW | 12 | population growth rate | 400 | |
| *Anabaena flos aquae* | Cyanobacterium | Non-vascular | FW | 5 | abundance | 38.8 | **38.8** |
| *Anabaena variabilis* | Cyanobacterium | Non-vascular | FW | 14 | population growth rate | 5.8 | 5.8 |
| *Ankistrodesmus* sp. | Green algae | Non-vascular | FW | 14 | population growth rate | 6 | 6 |
| *Apium nodiflorum* | European Marshwort | Vascular | FW | 14 | growth rate | 2.808 | 2.808 |
| *Ceramium tenuicorne* | Red Algae | Non-vascular | SW | 7 | population growth rate | 3.4 | 3.4 |
| *Chaetoceros gracilis* | Diatom | Non-vascular | SW | 3 | abundance | 36 | 36 |
| *Chara vulgaris* | Stonewort | Non-vascular | FW | 14 | chlorophyll | 4.033 | 4.033 |
| *Chlorella pyrenoidosa* | Green algae | Non-vascular | FW | 4 | population growth rate | 1.3 | 6.24 |
| *Chlorella pyrenoidosa* | Green algae | Non-vascular | FW | 14 | population growth rate | 30 | |
| *Chlorella* sp. | Green algae | Non-vascular | FW | 14 | population growth rate | 40 | 40 |
| *Chlorella vulgaris* | Green algae | Non-vascular | FW | 4 | population growth rate | 4.3 | 4.3 |
| *Chlorococcum hypnosporum* | Green algae | Non-vascular | FW | 4 | population growth rate | 25 | 25 |
| *Chlorococcum* sp. | Green algae | Non-vascular | FW | 14 | population growth rate | 5 | 5 |
| *Chroococcus* sp. | Cyanobacterium | Non-vascular | FW | 9 | abundance | 206 | 270.55 |
| *Chroococcus* sp. | Cyanobacterium | Non-vascular | FW | 9 | abundance | 218 | |
| *Chroococcus* sp. | Cyanobacterium | Non-vascular | FW | 9 | abundance | 441 | |
| *Coccolithus huxleyi* | Coccolithophorid | Non-vascular | SW | 3 | abundance | 2.26 | 2.26 |
| *Dictyosphaerium pulchellum* | Green algae | Non-vascular | FW | 14 | population growth rate | 6 | 6 |
| *Dunaliella tertiolecta* | Green algae | Non-vascular | FW | 4 | population growth rate | 4.9 | 44.60 |

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|---|---|---|---|---|---|---|---|
| *Dunaliella tertiolecta* | Green algae | Non-vascular | FW | 4 | population growth rate | 6.9 | |
| *Dunaliella tertiolecta* | Green algae | Non-vascular | FW | 4 | population growth rate | 300 | |
| *Dunaliella tertiolecta* | Green algae | Non-vascular | FW | 4 | population growth rate | 390 | |
| *Elodea nuttalli* | Waterweed, ditchmoss | Vascular | FW | 21 | biomass | 75 | 13.69 |
| *Elodea nuttalli* | Waterweed, ditchmoss | Vascular | FW | 21 | biomass | 75 | |
| *Elodea nuttalli* | Waterweed, ditchmoss | Vascular | FW | 21 | population change, general | 2.5 | |
| *Elodea nuttalli* | Waterweed, ditchmoss | Vascular | FW | 21 | population growth rate | 2.5 | |
| *Entomoneis punctulata* | Diatom | Non-vascular | SW | 3 | population growth rate | 24 | 24 |
| *Gracilaria tenuistipitata* | Red algae | Non-vascular | SW | 4 | population growth rate | 15 | 17.32 |
| *Gracilaria tenuistipitata* | Red algae | Non-vascular | SW | 4 | population growth rate | 20 | |
| *Hormidium flaccidum* | Green algae | Non-vascular | FW | 14 | population growth rate | 500 | 500 |
| *Lemna gibba* | Inflated duckweed | Vascular | FW | 14 | abundance | 27.3 | 27.3 |
| *Lemna minor* | Duckweed | Vascular | FW | 5 | abundance | 7 | 13.23 |
| *Lemna minor* | Duckweed | Vascular | FW | 7 | population growth rate | 25 | |
| *Lemna perpusilla* | Duckweed | Vascular | NR | 7 | population change, general | 15 | 15 |
| *Microcystis* sp. | Cyanobacterium | Non-vascular | FW | 9 | abundance | 120 | 197.95 |
| *Microcystis* sp. | Cyanobacterium | Non-vascular | FW | 9 | abundance | 162 | |
| *Microcystis* sp. | Cyanobacterium | Non-vascular | FW | 9 | abundance | 399 | |
| *Myriophyllum spicatum* | Eurasian watermilfoil | Vascular | SW | 28 | biomass | 137 | 56.98 |
| *Myriophyllum spicatum* | Eurasian watermilfoil | Vascular | SW | 35 | biomass | 137 | |
| *Myriophyllum spicatum* | Eurasian watermilfoil | Vascular | FW | 14 | growth rate | 5 | |
| *Myriophyllum spicatum* | Eurasian watermilfoil | Vascular | SW | 28 | photosynthesis | 80 | |
| *Myriophyllum spicatum* | Eurasian watermilfoil | Vascular | SW | 35 | photosynthesis | 80 | |
| *Navicula forcipata* | Diatom | Non-vascular | FW | 4 | population growth rate | 25 | 26.46 |

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|---|---|---|---|---|---|---|---|
| *Navicula forcipata* | Diatom | Non-vascular | FW | 4 | population growth rate | 28 | |
| *Navicula pelliculosa* | Diatom | Non-vascular | FW | 5 | abundance | 13.7 | 13.7 |
| *Nitzschia closterium* | Diatom | Non-vascular | SW | 3 | population growth rate | 17 | 17 |
| *Oscillatoria* sp. | Cyanobacterium | Non-vascular | FW | 14 | population growth rate | 40 | 40 |
| *Phaeodactylum tricornutum* | Diatom | Non-vascular | SW | 10 | abundance | 10 | 10 |
| *Potamogeton perfoliatus* | Pondweed | Vascular | SW | 28 | biomass | 25 | 42.52 |
| *Potamogeton perfoliatus* | Pondweed | Vascular | SW | 35 | biomass | 61 | |
| *Potamogeton perfoliatus* | Pondweed | Vascular | SW | 28 | photosynthesis | 45 | |
| *Potamogeton perfoliatus* | Pondweed | Vascular | SW | 35 | photosynthesis | 45 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | SW | 3 | abundance | 45 | 8.814 |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 4 | abundance | 0.7 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 4 | abundance | 2.4 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 5 | abundance | 67 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 3 | population growth rate | 10.5 | |
| *Pyrocystis lunula* | Dinoflagellate | Non-vascular | SW | 4 | abundance | 35000 | 35000 |
| *Scenedesmus acutus* | Green algae | Non-vascular | FW | 14 | population growth rate | 50 | 14.30 |
| *Scenedesmus acutus acutus* | Green Algae | Non-vascular | FW | 4 | population growth rate | 4.09 | |
| *Scenedesmus quadricauda* | Green algae | Non-vascular | FW | 4 | population growth rate | 2.7 | 11.62 |
| *Scenedesmus quadricauda* | Green algae | Non-vascular | FW | 14 | population growth rate | 50 | |
| *Scenedesmus subspicatus* | Green algae | Non-vascular | FW | 3 | population change, general | 36 | 36 |
| *Skeletonema costatum* | Diatom | Non-vascular | SW | 5 | abundance | 35.9 | 35.9 |
| *Spirodela polyrhiza* | Large duckweed | Vascular | NR | 7 | population change, general | 41 | 41 |
| *Spirulina platensis* | Cyanobacterium | Non-vascular | FW | 14 | population growth rate | 8.5 | 8.5 |
| *Stichococcus* sp. | Green algae | Non-vascular | FW | 14 | population growth rate | 1500 | 1500 |

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|---|---|---|---|---|---|---|---|
| *Synechococcus* sp. | Cyanobacterium | Non-vascular | SW | 3 | abundance | 0.55 | 14.57 |
| *Synechococcus* sp. | Cyanobacterium | Non-vascular | FW | 9 | abundance | 2 | |
| *Synechococcus* sp. | Cyanobacterium | Non-vascular | FW | 9 | abundance | 22 | |
| *Synechococcus* sp. | Cyanobacterium | Non-vascular | FW | 9 | abundance | 38 | |
| *Synechococcus* sp. | Cyanobacterium | Non-vascular | FW | 9 | abundance | 714 | |
| *Ulothrix fimbriata* | Green algae | Non-vascular | FW | 4 | population growth rate | 540 | 540 |
| *Zostera marina* | Eelgrass | Vascular | SW | 10 | photosynthesis | 3.2 | 3.2 |

[a]Data from ECOTOX database

## Irgarol

| Species | Common name | Species Group | Media Type | Duration (d) | Endpoint | $EC_{50}/IC_{50}$[a] (µg/L) | SMPV (µg/L) |
|---|---|---|---|---|---|---|---|
| *Anabaena flosaquae* | Cyanobacterium | Non-vascular | FW | 5 | abundance | 1.9 | 1.9 |
| *Apium nodiflorum* | European Marshwort | Vascular | FW | 14 | biomass | 0.01328 | 0.13 |
| *Apium nodiflorum* | European Marshwort | Vascular | FW | 14 | relative growth rate | 1.177 | |
| *Asterionella formosa* | Diatom | Non-vascular | FW | 4 | biomass | > 253 | > 253 |
| *Ceramium tenuicorne* | Red Algae | Non-vascular | SW | 7 | population growth rate | 0.96 | 0.96 |
| *Chaetoceros gracilis* | Diatom | Non-vascular | SW | 3 | abundance | 1.1 | 1.1 |
| *Chara vulgaris* | Stonewort | Non-vascular | FW | 14 | relative growth rate | 0.01175 | 0.01175 |
| *Chlamydomonas intermedia* | Green algae | Non-vascular | FW | 4 | biomass | 0.5 | 0.5 |
| *Chlorella vulgaris* | Green algae | Non-vascular | FW | 4 | biomass | 1.5 | 0.8857 |
| *Chlorella vulgaris* | Green algae | Non-vascular | FW | 5.3 | chlorophyll a | 0.523 | |
| *Chlorococcum* sp. | Green algae | Non-vascular | SW | 5 | abundance | 0.42 | 0.42 |
| *Chroococcus minor* | Cyanobacterium | Non-vascular | SW | 4 | population growth rate | 7.71 | 7.71 |

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|---|---|---|---|---|---|---|---|
| *Closterium ehrenbergii* | Green algae | Non-vascular | FW | 5 | population growth rate | 2.5 | 3 |
| *Closterium ehrenbergii* | Green algae | Non-vascular | FW | 5 | gamete production | 3.6 | |
| *Coccolithus huxleyi* | Coccolithophorid | Non-vascular | SW | 3 | abundance | 0.25 | 0.3012 |
| *Coccolithus huxleyi* | Coccolithophorid | Non-vascular | SW | 3 | population growth rate | 0.363 | |
| *Craticula accomoda* | Pennate diatom | Non-vascular | FW | 3 | biomass | 0.455 | 0.4770 |
| *Craticula accomoda* | Pennate diatom | Non-vascular | FW | 4 | biomass | 0.5 | |
| *Dunaliella tertiolecta* | Green algae | Non-vascular | FW | 4 | population growth rate | 0.9 | 0.9793 |
| *Dunaliella tertiolecta* | Green algae | Non-vascular | FW | 4 | population growth rate | 1.4 | |
| *Dunaliella tertiolecta* | Green algae | Non-vascular | FW | 4 | population growth rate | 0.73 | |
| *Dunaliella tertiolecta* | Green algae | Non-vascular | SW | 4 | population growth rate | 1 | |
| *Eisenia bicyclis* | Brown alga | Non-vascular | SW | 7 | cell cleavage | 2.2 | 2.717 |
| *Eisenia bicyclis* | Brown alga | Non-vascular | SW | 4 | size | 5.9 | |
| *Eisenia bicyclis* | Brown alga | Non-vascular | SW | 7 | size | 2 | |
| *Eisenia bicyclis* | Brown alga | Non-vascular | SW | 7 | size | 2.1 | |
| *Enteromorpha intestinalis* | Green algae | Non-vascular | SW | 3 | photosynthesis | 2.5 | 2.5 |
| *Fibrocapsa japonica* | Algae | Non-vascular | SW | 3 | population growth rate | 0.479 | |
| *Gracilaria tenuistipitata* | Red algae | Non-vascular | SW | 4 | population growth rate | 2 | 2 |
| *Lemna gibba* | Inflated duckweed | Vascular | FW | 7 | size | 11 | 4.195 |
| *Lemna gibba* | Inflated duckweed | Vascular | FW | 14 | abundance | 1.6 | |
| *Lemna minor* | Duckweed | Vascular | FW | 7 | size | 8.1 | 8.1 |
| *Myriophyllum spicatum* | Eurasian watermilfoil | Vascular | FW | 14 | relative growth rate | 2 | 2 |
| *Myriophyllum verticillatum* | Whorl-leaf watermilfoil | Vascular | FW | 43 | length | 2.3 | 1.965 |
| *Myriophyllum verticillatum* | Whorl-leaf watermilfoil | Vascular | FW | 43 | weight | 1.1 | |
| *Myriophyllum verticillatum* | Whorl-leaf watermilfoil | Vascular | FW | 43 | biomass | 3 | |

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|---|---|---|---|---|---|---|---|
| *Navicula forcipata* | Diatom | Non-vascular | FW | 4 | population growth rate | 0.5 | 0.5916 |
| *Navicula forcipata* | Diatom | Non-vascular | FW | 4 | population growth rate | 0.7 | |
| *Navicula pelliculosa* | Diatom | Non-vascular | FW | 5 | abundance | 0.1 | 0.1 |
| *Nitzschia* sp. | Diatom | Non-vascular | FW | 4 | biomass | 0.8 | 0.8 |
| *Pediastrum duplex* | Green algae | Non-vascular | FW | 3 | biomass | 2.4 | 2.4 |
| *Porphyra yezoensis* | Red algae | Non-vascular | SW | 4 | size | 0.1 | 1.206 |
| *Porphyra yezoensis* | Red algae | Non-vascular | SW | 4 | size | 0.4 | |
| *Porphyra yezoensis* | Red algae | Non-vascular | SW | 4 | size | 1.3 | |
| *Porphyra yezoensis* | Red algae | Non-vascular | SW | 4 | germination | 2.7 | |
| *Porphyra yezoensis* | Red algae | Non-vascular | SW | 4 | germination | 3.6 | |
| *Porphyra yezoensis* | Red algae | Non-vascular | SW | 4 | germination | 6.1 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 3 | abundance | 1.47 | 3.584 |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 3 | abundance | 1.6 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | SW | 3 | abundance | 10 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 3 | abundance | 10.8 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 5 | abundance | 1.3 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 3 | population growth rate | 10 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 3 | population growth rate | 2.3 | |
| *Ruppia maritima* | Widgeon-grass | Vascular | SW | 28 | biomass | 1.8872 | 1.607 |
| *Ruppia maritima* | Widgeon-grass | Vascular | SW | 28 | biomass | 1.9228 | |
| *Ruppia maritima* | Widgeon-grass | Vascular | SW | 28 | growth, general | 0.8425 | |
| *Ruppia maritima* | Widgeon-grass | Vascular | SW | 28 | morphology, general | 2.008 | |
| *Ruppia maritima* | Widgeon-grass | Vascular | SW | 28 | vegetative reproduction | 1.7484 | |
| *Scenedesmus acutus* | Green algae | Non-vascular | FW | 4 | biomass | 3.3 | 4.102 |

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|---|---|---|---|---|---|---|---|
| *Scenedesmus acutus* | Green algae | Non-vascular | FW | 4 | biomass | 5.1 | |
| *Scenedesmus subspicatus* | Green algae | Non-vascular | FW | 3 | abundance | 2.4 | 2.4 |
| *Skeletonema costatum* | Diatom | Non-vascular | SW | 5 | abundance | 0.45 | 0.3612 |
| *Skeletonema costatum* | Diatom | Non-vascular | SW | 4 | population growth rate | 0.29 | |
| *Staurastrum sebaldi* | Desmid | Non-vascular | FW | 4 | biomass | 2.5 | 2.5 |
| *Stuckenia pectinata* | Sago Pondweed | Vascular | SW | 28 | biomass | 6.1152 | 6.757 |
| *Stuckenia pectinata* | Sago Pondweed | Vascular | SW | 28 | biomass | 7.4664 | |
| *Synechococcus* sp. | Blue-green algae | Non-vascular | SW | 3 | abundance | 0.16 | 0.16 |
| *Tetraselmis* sp. | Green flagellate | Non-vascular | SW | 3 | population growth rate | 0.1 | 0.1 |
| *Thalassiosira pseudonana* | Diatom | Non-vascular | SW | 4 | population growth rate | 0.41 | 0.41 |
| *Thalassiosira weissflogii* | Diatom | Non-vascular | SW | 3 | population growth rate | 0.28 | 0.28 |
| *Zostera marina* | Eelgrass | Vascular | SW | 10 | biomass | 1.1 | 1.465 |
| *Zostera marina* | Eelgrass | Vascular | SW | 10 | distance | 2.6 | |
| *Zostera marina* | Eelgrass | Vascular | SW | 10 | photosynthesis | 1.1 | |

[a]Data from ECOTOX database

## Metolachlor

| Species | Common name | Species group | Medium | Duration (d) | Endpoint | $EC_{50}/IC_{50}$[a] (µg/L) | SMPV (µg/L) |
|---|---|---|---|---|---|---|---|
| *Anabaena cylindrica* | Cyanobacterium | Non-vascular | FW | 3 | abundance | 5000 | > 5000 |
| *Anabaena flos aquae* | Cyanobacterium | Non-vascular | FW | 5 | abundance | 1200 | 1200 |
| *Ceratophyllum demersum* | Coon-tail | Vascular | FW | 14 | biomass | 70 | 70 |
| *Chlamydomonas reinhardtii* | Green algae | Non-vascular | FW | 4 | chlorophyll | 1138 | 1138 |
| *Chlorella fusca* | Green algae | Non-vascular | FW | 12 | population growth rate | 100.61 | 105.27 |

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|---|---|---|---|---|---|---|---|
| *Chlorella fusca* | Green algae | Non-vascular | FW | 12 | population growth rate | 105.03 | |
| *Chlorella fusca* | Green algae | Non-vascular | FW | 12 | population growth rate | 107.3 | |
| *Chlorella fusca* | Green algae | Non-vascular | FW | 12 | population growth rate | 108.3 | |
| *Chlorella pyrenoidosa* | Green algae | Non-vascular | FW | 4 | population growth rate | 12717.2 | 12717 |
| *Chlorella vulgaris* | Green algae | Non-vascular | FW | 4 | population growth rate | 18926.1 | 1960.1 |
| *Chlorella vulgaris* | Green algae | Non-vascular | FW | 4 | chlorophyll | 203 | |
| *Elodea canadensis* | Waterweed | Vascular | FW | 14 | biomass | 2355 | 2355 |
| *Lemna gibba* | Inflated duckweed | Vascular | FW | 14 | abundance | 48 | 67.42 |
| *Lemna gibba* | Inflated duckweed | Vascular | FW | 7 | abundance | 304 | |
| *Lemna gibba* | Inflated duckweed | Vascular | FW | 5 | abundance | 21 | |
| *Lemna minor* | Duckweed | Vascular | FW | 4 | abundance | 360 | 351.4 |
| *Lemna minor* | Duckweed | Vascular | FW | 4 | population growth, general | 343 | |
| *Microcystis* sp. | Cyanobacterium | Non-vascular | FW | 4 | chlorophyll | 3000 | > 3000 |
| *Myriophyllum heterophyllum* | Two-leaf water-milfoil | Vascular | FW | 14 | biomass | 3000 | > 3000 |
| *Myriophyllum sibiricum* | Water milfoil | Vascular | FW | 14 | area | 579.6 | 1024.4 |
| *Myriophyllum sibiricum* | Water milfoil | Vascular | FW | 14 | growth rate | 1535.2 | |
| *Myriophyllum sibiricum* | Water milfoil | Vascular | FW | 14 | length | 670.1 | |
| *Myriophyllum sibiricum* | Water milfoil | Vascular | FW | 14 | length | 1896 | |
| *Myriophyllum sibiricum* | Water milfoil | Vascular | FW | 14 | number of roots | 1684.8 | |
| *Myriophyllum sibiricum* | Water milfoil | Vascular | FW | 14 | weight | 606.7 | |
| *Najas* sp. | Water nymph | Vascular | FW | 14 | biomass | 242 | 242 |
| *Navicula pelliculosa* | Diatom | Non-vascular | FW | 5 | abundance | 380 | 380 |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 4 | abundance | 5508.1* | 34.16 |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 4 | abundance | 50.9 | |

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|---|---|---|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 4 | abundance | 55.5 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 5 | abundance | 10 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 4 | chlorophyll | 84 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 4 | population growth, general | 77 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 3 | abundance | 37.17 | |
| *Pseudokirchneriella subcapitata* | Green algae | Non-vascular | FW | 5 | abundance | 8 | |
| *Salvinia natans* | Floating watermoss (fern) | Vascular | FW | 28 | growth rate | 150 | 86.60 |
| *Salvinia natans* | Floating watermoss (fern) | Vascular | FW | 28 | biomass | 50 | |
| *Scenedesmus quadricauda* | Green algae | Non-vascular | FW | 4 | population growth rate | 600 | 600 |
| *Scenedesmus vacuolatus* | Green algae | Non-vascular | FW | 1 | abundance | 232 | 232 |
| *Scenedesmus subspicatus* | Green algae | Non-vascular | FW | 3 | abundance | 57100 | 57100 |
| *Skeletonema costatum* | Diatom | Non-vascular | SW | 5 | abundance | 61 | 81.91 |
| *Skeletonema costatum* | Diatom | Non-vascular | SW | 5 | abundance | 110 | |

*Not used in SMPV
[a]Data from ECOTOX database

## Pentachlorophenol

| Species | Common name | Species Group | Media Type | Duration (d) | Endpoint | $EC_{50}$[a] (µg/L) | SMPV (µg/L) |
|---|---|---|---|---|---|---|---|
| *Anabaena flos aquae* | Cyanobacterium | Non-vascular | FW | 5 | abundance | 50 | 50 |
| *Anabaena inaequalis* | Cyanobacterium | Non-vascular | FW | 4 | abundance | 130 | 130 |
| *Callitriche platycarpa* | Water Starwort | Vascular | FW | 21 | relative growth rate | 3300 | 3300 |
| *Callitriche platycarpa* | Water Starwort | Vascular | FW | 21 | dry biomass | 3300 | |
| *Chlamydomonas reinhardtii* | Green Algae | Non-vascular | FW | 10 | population change, general | 360 | 327.92 |

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|---|---|---|---|---|---|---|---|
| *Chlamydomonas reinhardtii* | Green Algae | Non-vascular | FW | 3 | population change, general | 168 | |
| *Chlamydomonas reinhardtii* | Green Algae | Non-vascular | FW | 4 | population change, general | 405 | |
| *Chlamydomonas reinhardtii* | Green Algae | Non-vascular | FW | 7 | population change, general | 410 | |
| *Chlamydomonas reinhardtii* | Green Algae | Non-vascular | FW | 10 | population growth rate | 360 | |
| *Chlamydomonas reinhardtii* | Green Algae | Non-vascular | FW | 3 | population growth rate | 220 | |
| *Chlamydomonas reinhardtii* | Green Algae | Non-vascular | FW | 4 | population growth rate | 410 | |
| *Chlamydomonas reinhardtii* | Green Algae | Non-vascular | FW | 7 | population growth rate | 410 | |
| *Chlamydomonas* sp. | Green Algae | Non-vascular | SW | 4 | growth rate | 1400 | 1400 |
| *Chlorella emersonii* | Green Algae | Non-vascular | FW | 20 | population growth rate | 5000 | 5000 |
| *Chlorella kessleri* | Green Algae | Non-vascular | FW | 4 | abundance | 34300 | 34300 |
| *Chlorella pyrenoidosa* | Green Algae | Non-vascular | SW | 4 | growth rate | 5500 | 4134.53 |
| *Chlorella pyrenoidosa* | Green Algae | Non-vascular | FW | 4 | growth rate | 7000 | |
| *Chlorella pyrenoidosa* | Green Algae | Non-vascular | FW | 2 | chlorophyll a | 2300 | |
| *Chlorella pyrenoidosa* | Green Algae | Non-vascular | FW | 6 | population growth rate | 3300 | |
| *Chlorella vulgaris* | Green Algae | Non-vascular | FW | 20 | abundance | 10030 | 7737.24 |
| *Chlorella vulgaris* | Green Algae | Non-vascular | FW | 4 | growth rate | 10300 | |
| *Chlorella vulgaris* | Green Algae | Non-vascular | FW | 4 | growth rate | 10300 | |
| *Chlorella vulgaris* | Green Algae | Non-vascular | FW | 20 | population growth rate | 12000 | |
| *Chlorella vulgaris* | Green Algae | Non-vascular | FW | 7 | population growth rate | 1663 | |
| *Chlorella vulgaris var. viridis* | Green Algae | Non-vascular | NR | 2 | population growth rate | 10120.92 | |
| *Chlorella vulgaris var. viridis* | Green Algae | Non-vascular | NR | 3 | population growth rate | 7723.86 | |
| *Chlorella zofingiensis* | Green Algae | Non-vascular | NR | 2 | chlorophyll | 42.6144 | 42.61 |
| *Dunaliella* sp. | Green Algae | Non-vascular | SW | 4 | growth rate | 3600 | 3600 |
| *Dunaliella tertiolecta* | Green Algae | Non-vascular | SW | 4 | abundance | 170 | 170 |

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|---|---|---|---|---|---|---|---|
| *Elodea canadensis* | Waterweed | Vascular | FW | 21 | length | 4 | |
| *Elodea canadensis* | Waterweed | Vascular | FW | 21 | dry biomass | 3265 | 3265 |
| *Elodea nuttalli* | Waterweed, Ditchmoss | Vascular | FW | 21 | dry weigth | 109 | 333.1096 |
| *Elodea nuttalli* | Waterweed, Ditchmoss | Vascular | FW | 21 | dry biomass | 1018 | |
| *Lemna gibba* | Inflated Duckweed | Vascular | FW | 14 | abundance | 250 | 413.9593 |
| *Lemna gibba* | Inflated Duckweed | Vascular | FW | 7 | reproduction, general | 532.68 | |
| *Lemna gibba* | Inflated Duckweed | Vascular | FW | 7 | vegetative reproduction | 532.68 | |
| *Lemna minor* | Duckweed | Vascular | FW | 10 | photosynthesis | 1670 | 849.38 |
| *Lemna minor* | Duckweed | Vascular | FW | 4 | photosynthesis | 1940 | |
| *Lemna minor* | Duckweed | Vascular | FW | 2 | abundance | 800 | |
| *Lemna minor* | Duckweed | Vascular | FW | 10 | population change, general | 1250 | |
| *Lemna minor* | Duckweed | Vascular | FW | 4 | population change, general | 610 | |
| *Lemna minor* | Duckweed | Vascular | FW | 3 | survival | 190 | |
| *Lemna trisulca* | Duckweed | Vascular | FW | 21 | relative growth rate | 1282 | 1457.05 |
| *Lemna trisulca* | Duckweed | Vascular | FW | 21 | dry biomass | 1656 | |
| *Macrocystis pyrifera* | Giant Kelp | Non-vascular | SW | 4 | photosynthesis | 300 | 300 |
| *Myriophyllum spicatum* | Eurasian Watermilfoil | Vascular | FW | 21 | dry weigth | 236 | 614.30 |
| *Myriophyllum spicatum* | Eurasian Watermilfoil | Vascular | FW | 21 | dry biomass | 1599 | |
| *Navicula pelliculosa* | Diatom | Non-vascular | FW | 5 | abundance | 124 | 124 |
| *Pavlova* sp. | Chrysophyte | Non-vascular | SW | 4 | growth rate | 200 | 200 |
| *Phaeodactylum tricornutum* | Diatom | Non-vascular | SW | 4 | growth rate | 3000 | 3000 |
| *Potamogeton crispus* | Curled Pondweed | Vascular | FW | 21 | relative growth rate | 338 | 416 |
| *Potamogeton crispus* | Curled Pondweed | Vascular | FW | 21 | dry biomass | 512 | |

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|---|---|---|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 3 | abundance | 240 | 259.42 |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 3 | population growth rate | 100 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 3 | population growth rate | 250 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 4 | growth rate | 110 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 4 | growth rate | 150 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 4 | growth rate | 760 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 4 | growth rate | 420 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 4 | growth rate | 420 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 2 | abundance | 410 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 4 | abundance | 70 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 4 | abundance | 290 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 4 | abundance | 290 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 5 | abundance | 50 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 3 | chlorophyll a | 412 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 5 | chlorophyll a | 335 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 7 | chlorophyll a | 331 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 4 | population change, general | 310 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 5 | population change, general | 520 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 4 | population growth rate | 312 | |
| *Pseudokirchneriella subcapitata* | Green Algae | Non-vascular | FW | 5 | population growth rate | 518 | |
| *Ranunculus longirostris* | Longbeak Buttercup | Vascular | FW | 21 | length | 341 | 449.30 |
| *Ranunculus longirostris* | Longbeak Buttercup | Vascular | FW | 21 | dry biomass | 592 | |
| *Ranunculus peltatus* | Pond Water Crowfoot | Vascular | FW | 21 | dry weight | 16 | 121.46 |
| *Ranunculus peltatus* | Pond Water Crowfoot | Vascular | FW | 21 | dry biomass | 922 | |

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|---|---|---|---|---|---|---|---|
| *Scenedesmus abundans* | Green Algae | Non-vascular | FW | 4 | growth rate | 90 | 90 |
| *Scenedesmus quadricauda* | Green Algae | Non-vascular | FW | 4 | growth rate | 80 | 80 |
| *Scenedesmus subspicatus* | Green Algae | Non-vascular | FW | 3 | population growth rate | 183 | 183 |
| *Skeletonema costatum* | Diatom | Non-vascular | SW | 4 | abundance | 80 | 35.26 |
| *Skeletonema costatum* | Diatom | Non-vascular | SW | 4 | abundance | 20.3 | |
| *Skeletonema costatum* | Diatom | Non-vascular | SW | 5 | abundance | 27 | |
| *Thalassiosira pseudonana* | Diatom | Non-vascular | SW | 4 | abundance | 179 | 179 |

[a]Data from ECOTOX database