EPA Public Access

Author manuscript

Environ Pollut. Author manuscript; available in PMC 2019 July 01.

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Published in final edited form as:

Environ Pollut. 2018 July ; 238: 270–280. doi:10.1016/j.envpol.2018.03.003.

Aquatic plants: test species sensitivity and minimum data requirement evaluations for chemical risk assessments and aquatic life criteria development for the USA (version before eliminating tables and figures)

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Abstract

Phytotoxicity results from the publicly-available ECOTOX database were summarized for 20 chemicals and 188 aquatic plants to determine species sensitivities and the ability of a species-limited toxicity database to serve as a surrogate for a larger database. The lowest effect concentrations reducing the sublethal response parameter of interest by 50% relative to the controls (EC₅₀) usually varied several orders of magnitude for the 119 freshwater and 69 saltwater plants exposed to the same test chemicals. Generally, algae were more sensitive than floating and benthic species but inter-specific differences for EC₅₀ values were sometimes considerable within and between phyla and no consistently sensitive species was identified for the morphologically-diverse taxa. Consistent equivalencies of the phytotoxicity databases for freshwater-saltwater plants and floating-benthic macrophyte species were not demonstrated. Two species-sensitivity distribution plots (SSDs) were constructed for each of the 20 chemicals, one based on all available phytotoxicity information (range=10-76 test species) and another based on information for only five species recommended for pesticide hazard evaluations. HC₅ values (hazardous concentration to 5% of test species) estimated from the two SSDs usually differed four-fold or less for the same chemical. HC₅ values for the five species were often less than those for the more species-populated data sets. Consequently, the collective response of the five test species shows promise as an interim aquatic plant minimum data requirement for aquatic life criteria development. In contrast, the individual EC₅₀ values for the five species were a less conservative platform than HC₅ values for calculation of the Final Plant Value needed for the same process. The conclusions may differ for comparisons based on other test chemicals, test species, response parameters and calculations.

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Keywords

Aquatic plants; Phytotoxicity; Interspecific sensitivities; Minimum data requirement

1. Introduction

Aquatic plants, broadly defined, undergo chloroxygenic photosynthesis (Bolton 2016). They are a diverse group of freshwater and saltwater species with varied life cycles and architectures. The single cell and multicellular species can be attached, free-floating, submerged or emergent and are represented in the Kingdoms Plantae, Protista, Eubacteria and Chromista. Although their total diversity is unknown, they are numerous. Estimates of algal diversity alone range from 30,000 to 1,000,000 species (Guiry 2012) which includes, among others, as many as 100,000 to 200,000 diatoms (Hawksworth and Kalin-Arroyo, 1995; Guiry, 2012), 10,000 seaweed species (www.seaweed.ie/seaweeds.php) and 2,000 to 8,000 species of cyanobacteria (Nabout et al., 2013).

It is well known that aquatic plants have ecological and economic value. They serve as the base of most aquatic food chains, provide shelter, stabilize sediment, protect shorelines and act as carbon sinks. In addition, they serve as indicators of water quality and provide filtration and detoxification of anthropogenic chemicals. Marine plants produce 70% to 80% of atmospheric oxygen (Hall, 2011). The economic value of the goods and services produced by coastal shallow -water vegetated ecosystems can be significant (Duarte et al., 2008; Barbier et al., 2011). The many reported global estimates of their annual economic value range between millions to trillions USD. For example, the worldwide commercial value of seaweeds for human consumption alone is between \$5 and \$6 billion/yr (www.fao.org/docrep/006/y4765e/y4765e04.htm). In contrast to their benefits, excessive growth from endemic and invasive plant species can impact human health, irrigation, fish and wildlife, recreation and navigation. The estimated cost of controlling invasive plant growth in the U.S. is at least \$100 million/yr (Pimentel et al., 2005).

The role of aquatic plants for decisions concerning the environmental impact of chemicals has been debated for almost 40 years with no resolution. Understanding the toxicities of anthropogenic chemicals to aquatic plants has been slow to advance and baseline toxicity research has not kept pace with that for aquatic animal toxicity research. Aquatic plants, despite recognition of their toxicological importance since 1967 (USEPA, 1982), have seldom been a determining factor for chemical risk assessments other than for a few agriculture herbicides and marine anti-foulant coatings. They have been of secondary importance to faunal species for regulatory decisions related to, among others, the Clean Water Act (1972), Toxic Substances Control Act (1976), the Comprehensive Environmental Response, Compensation, and Liability Act (1980) and the NPDES permitting process (National Pollutant Discharge Elimination System). This usual minor role is attributable to a faunal-sensitivity bias and to uncertainty concerning which plant species or group of species are chemically sensitive and if their sensitivity is representative of that for the aquatic plant community at large. The faunal bias is due to a lingering assumption, beginning with Kenaga and Moolenaar (1979) and reinforced by Stephan et al. (1985), that aquatic plants are

generally less sensitive than animal species to chemicals. This assumption has been refuted (Lewis, 1995; Wang and Freemark, 1995; Lytle and Lytle, 2001) but the perception of general insensitivity persists.

Aquatic plants have been an almost non-factor for most National, state and tribal water quality criteria for aquatic life developed during the past 30 years in the U.S. This situation is due, in part, to an absence of clarity for their use in the process. The original methodology (Stephan et al., 1985) includes calculation of an acute criterion which is based on at least one faunal species for at least eight different taxonomic families for acute toxicity and three different families for chronic toxicity. There are no similar specific taxonomic requirements for aquatic plants and acute toxicity (algicidal or phytocidal concentrations). A chronic criterion is also needed for criteria development and it is the most sensitive of either the faunal-based Final Chronic Value (FCV) or the Final Plant Value (FPV). The FPV is the effect concentration resulting from a 96-h toxicity test conducted with an unspecified alga and/or from a chronic test conducted with an unspecified aquatic vascular plant. It is uncertain if the results from one or two phytotoxicity tests is sufficient to represent the sensitivity of the diverse aquatic plant community or if a HC₅ value (hazardous concentration to 5% of test species) from a species sensitivity distribution plot is more appropriate as recommended for aquatic faunal toxicity data. In practice, the FCV is usually the decision calculation due to the limited availability of phytotoxicity information.

The consequence of the secondary role of aquatic plants for most chemical risk assessments and aquatic life criteria is unknown but important to identify to ensure environmental protection for primary producers. One step for reducing uncertainty is identification of either a minimum data requirement (MDR) based on a list of sensitive plant species representing various taxonomic groups or analysis of species sensitivity distributions (SSDs). The primary objective of this report is to provide insight on the value of both options. A subset of the Ecotoxicology Database (ECOTOX; <http://cfpub.epa.gov/ecotox/>) was summarized to identify sensitive aquatic plant species based on EC₅₀ concentrations (concentration reducing the response parameter of interest by 50% relative to the control). In addition, as a technical expansion of Thursby and Lewis (2013), the database was used as a platform to determine the ability of a species-limited data set to serve as a surrogate for larger species-populated data sets based on HC₅ value comparisons.

2. Materials and methods

A brief description of methods follow. More detailed information for data selection, data standardization, data analyses, and lognormal probability plots appears in Thursby and Lewis (2015).

2.1. Database selection

The publicly accessible ECOTOX database was the primary source of phytotoxicity information for the review. It is the main source of toxicity information for current U.S. recommended water quality criteria for aquatic life and is a searchable on-line USEPA system that includes information for about 10,300 chemicals and 10,500 aquatic and terrestrial species dating back to 1915. It has minimum data requirements related to the

chemical, test species, response parameters, dose, and exposure duration. Toxicity data used from ECOTOX was checked for accuracy against the original citations. Secondary sources of toxicity information included Bao et al. (2011), Chalifour and Juneau (2011), Larras et al. (2012), U.S. Geological Survey, Organization for Economic Cooperation and Development and the USEPA's Office of Pesticide Program's data evaluation records.

The phytotoxicity databases for 38 chemicals were reviewed and those for 20 chemicals, dominated by herbicides and heavy metals, were chosen for analysis (Table 1). The 20 chemicals were selected based on the requirement that toxicity information was needed for at least ten test species of which five species ideally were those recommended for pesticide toxicity screening (FIFRA-Federal Insecticide, Fungicide and Rodenticide Act). These species are the freshwater green microalga, *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*), *Anabaena flos-aquae* (freshwater cyanobacterium), *Navicula pelliculosa* (freshwater pennate diatom), *Skeletonema costatum* (saltwater centric diatom) and *Lemna gibba* (floating duckweed). Toxicity information was available for the five species except for As, Cr, Pb, Ni and Zn for which one (As, Cr), two (Ni, Zn) and three (Pb) substitute species were used. The sequence of substitute selection was a test species from the same genus, and, if not available, then the same order and class. The list of substitutes used for each chemical is available from Thursby and Lewis (2015). The FIFRA-recommended five species data set was chosen for comparison due to the availability of a relatively large toxicity database derived using standard and widely-used toxicity test protocols (www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines).

2.2. Database homogeneity

The ECOTOX phytotoxicity information was screened for experimental consistency prior to use. Toxicity results not expressed as effect (EC₅₀), inhibitory (IC₅₀) and lethal (LC₅₀) concentrations were not used. EC₅₀ and IC₅₀ concentrations are often used interchangeably in phytotoxicity testing evaluations. LC₅₀ values represent the concentration lethal to 50% of the exposed test species. Most results were expressed as an EC₅₀ value since many phytotoxicity tests are designed for its calculation and it is the recommended calculation for the FIFRA-recommended test species. Results reported as greater than (>) were included as absolute values but the greater than was retained with the resulting geometric mean. The EC₅₀ values were based usually on growth-related effects. Results based on non-standard endpoints such as enzyme concentrations and non-chlorophyll *a* pigment concentrations were not used. When more than one toxic effects concentration for the same endpoint (for the same species) was available within the standardized data sets described above, the geometric mean of those data was used for that endpoint. When toxic effects values for more than one endpoint were available for the same species, the endpoint with the lowest value represented the sensitivity of that species. Results for vascular and non-vascular freshwater and saltwater species were combined for the SSD plots. The Integrated Taxonomic Information System (www.itis.gov) was used as the authority for species names. No distinction was made between static and renewal techniques. The exposure durations were limited to three to five days for microalgal tests; 7 to 14 days for duckweed toxicity tests and 14 to 35 days for tests conducted with other vascular plants. An exception, two day durations

were included when an endpoint such as seaweed spore germination was available. One of the limitations of plant data sets is the common lack of measured test concentrations. To maximize the taxonomic representation within the data sets, EC₅₀ values based on measured and unmeasured chemistry were included in the analyses.

2.3 Species-sensitivity comparisons

The EC₅₀ values for each of the 20 test chemicals were compared between freshwater and saltwater taxa and between and among algae, duckweeds, and benthic macrophytes (attached, submersed, emergent taxa). It is important to note that the interspecific EC₅₀ comparisons, despite our attempt for experimental homogeneity, were sometimes based on results derived from different techniques. This condition is common for comparisons of this type due to a lack of consensus phytotoxicity test methods for all but a few plant test species.

2.4. Species-sensitivity distribution plots (SSD)

Toxicity information for the 20 chemicals were separated into data sets for all test species and for only the five FIFRA-recommended test species. Separate SSD plots and HC₅ values were determined for each data set assuming a log-normal distribution. All SSD plots appear in Thursby and Lewis (2015). Parameters for linear regressions are in Table 3. Proportional rank was calculated as $R/(N+1)$, where R was the cumulative rank of the datum and N was the total number of values in the SSD. The goodness of fit to the log-normal distribution was judged graphically from a linearized form of the distribution. Regression parameters were calculated using standard least square regression analysis. For atrazine, chromium, diuron, irgarol, metribuzin, and pentachlorophenol, there was a clear separation of the “linearized” data into higher and lower concentration subsets. For these compounds, only the lower concentration half of the data were used.

The linearized form of the distribution used was from Lee and Wang (2003). The cumulative distribution function for a log-normal plot is:

$$F(c) = \Phi\left(\frac{c - \mu}{\sigma}\right) \quad \text{Equation 1}$$

Where:

$F(c)$ = proportion of values less than or equal to c , i.e., the proportional rank;

c = in our case is the ln of a species EC₅₀ value from the SSD;

μ = arithmetic mean of all EC₅₀ values in the statistical population modeled by the distribution;

σ = the standard deviation of all EC₅₀ values in the statistical population; and

Φ = the standard normal distribution function, which calculates the percentage of values that are less than or equal to c . In Microsoft Excel, this function is *NORMSDIST*.

Applying the inverse of the standard normal distribution function (*NORMSINV* in Excel) to both sides of the above equation results in:

$$\Phi^{-1}F(c) = \left(\frac{c - \mu}{\sigma} \right) \quad \text{Equation 2}$$

$$c = \sigma \cdot \Phi^{-1}F(c) + \mu \quad \text{Equation 3}$$

This is a linear function between $\Phi^{-1}F(c)$ on the x-axis and c on the y-axis. The slope and intercept of the equation fit to the data give an estimate of the parameters needed for the log normal distribution model of the data. Solving the equation for $F(c)$ equal to any proportion of interest gives the value of c equivalent to the concentration affecting that proportion of the species. In practice, 0.05 is usually the proportion of interest (the 5th percentile) and represents the concentration affecting 5% of the species (HC₅ value). The HC₅ value is considered an estimate of the “true” or plant community threshold effect concentration. The HC₅ values and confidence limits were determined for all test species and five test species only data sets using Equation 3.

3. Results and discussion

3.1. Phytotoxicity database

The number of test species, the lowest EC₅₀ values and corresponding test species for each chemical appear in Table 1. All EC₅₀ values for each test species and each chemical are listed in Thursby and Lewis (2015). Test species diversity was uneven across test chemicals. The larger databases were for Cu (76 species), atrazine (triazine herbicide, 69 species), irgarol (anti-fouling algicide, 48 species) and diuron (broad spectrum herbicide, 46 species). Toxicity results were available for a total of 188 test species with varied morphologies (examples, Fig. 1). Toxicity results for algae dominated the database; 81% freshwater and 92% saltwater. Freshwater test species (total=119) were more numerous than saltwater test species (total=69). On average, 78 (range=20-96) % of the EC₅₀ values for each test chemical was for freshwater species. The most saltwater toxicity data was for As, Cu and Pb (55% to 80% of results). Only one saltwater EC₅₀ value was available for seven test chemicals (Table 2). The paucity of information for saltwater plants observed here is not unusual and in need of correction (Lytle and Lytle 2001; Lewis and Devereux 2009).

The EC₅₀ values (µg L⁻¹) for all chemicals and all freshwater species were between 0.013 and 446,000 and between 0.1 and 48,900 for all saltwater species (Table 2). Phytotoxicities in decreasing order based on lowest EC₅₀ values for all freshwater test species were: irgarol>Cu>diquat>prometryn=triclosan>diuron>linuron>pentachlorophenol>Zn>terbuthylazine>metribuzin>Cd>atrazine>metolachlor>Se>Cr>As>Ni>Pb>glyphosate. Phytotoxicities for all saltwater species in declining order were: irgarol>Cu>As>diuron>triclosan>metribuzin>atrazine>Pb>terbuthylazine>pentachlorophenol>linuron>Cr>prometryn>Zn>Cd>metolachlor>Ni>Se>glyphosate>diquat.

3.2. Interspecific sensitivity differences

In general terms, sensitivities in declining order were: algae>submersed/emergent vascular species> floating duckweeds but no consistently sensitive single test species was apparent. EC₅₀ values were chemical-specific and varied sometimes orders of magnitude between species representing the same and different phyla. EC₅₀ values for all test species exposed to the same test chemical ranged from 10 (triclosan)-21,233 (Cu) and 2 (glyphosate)-2,346 (Zn) for the five FIFRA-recommended test species. Sensitivity differences between freshwater and saltwater algal species were mixed. These differences, as well as others, are discussed in greater detail below.

3.3. Microalgae

EC₅₀ values varied considerably for the algal test species. For example, the range of EC₅₀ values ($\mu\text{g L}^{-1}$) for sensitive freshwater algae (Table 1) was 0.013 (irgarol) to 3,530 (glyphosate). EC₅₀ values for different algal species within the same phylum and between different phyla exposed to the same chemical also differed (Table 4). EC₅₀ differences for freshwater species representing the same phylum were approximately between 6 (irgarol) and 2,240 (Cu) for green microalgae, 4 (metolachlor) and 412 (atrazine) for cyanobacteria and between 83 (Cu) and 25,300 (irgarol) for diatoms. EC₅₀ differences for saltwater diatoms ranged approximately between 2 (atrazine) and 12,833 (Cu).

Algae were usually the most sensitive test species (Table 1). For freshwater species this included six green spp. (nine chemicals), four cyanobacteria or blue-green spp. (four chemicals) and two diatoms (two chemicals). The most sensitive saltwater species were usually diatoms (five spp.; 13 chemicals). Of these, the centric diatom, *Skeletonema costatum*, was commonly the most sensitive species (nine chemicals) but usually because it was the only saltwater test species (seven of the nine chemicals). The FIFRA-recommended algae for screening pesticide toxicity, *A. flos-aquae*, *N. pelliculosa*, *R. subcapitata* and *S. costatum*, were the most sensitive test species to 11 chemicals (Table 1). The most sensitive freshwater and saltwater test species to Pb, metribuzin and terbuthylazine were both FIFRA-recommended taxa.

3.4. Duckweeds

EC₅₀ values were available for all chemicals and at least one of eight duckweeds: *Lemna aequinoctialis* (lesser duckweed), *Lemna gibba* (fat duckweed), *Lemna minor* (common duckweed), *Lemna perpusilla* (minute duckweed), *Lemna trisulca* (star duckweed), *Spirodella polyrrhiza* (greater duckweed), *Spirodella punctata* (formerly *Landoltia punctata*-dotted duckweed) and *Wolffia globosa* (Asia watermeal) (Table 5). The more duckweed-diverse data sets were for Cu, Cd, diuron and prometryn. EC₅₀ values ($\mu\text{g L}^{-1}$) for the four or five species exposed to these four toxicants ranged from 82.6 to 3,300 (Cu), 54.1 to 800 (Cd), 9.3 to 41 (diuron) and 11.8 to 85 (prometryn), respectively. The more common test species were *L. minor* (13 chemicals) and *L. gibba* (13 chemicals). The EC₅₀ values ($\mu\text{g L}^{-1}$) for the 13 chemicals ranged from 4 (diquat) to 9,600 (Zn) (*L. minor*) and 1.6 (irgarol) to 12,400 (glyphosate) (*L. gibba*). Both species were exposed to the same seven test chemicals. *L. gibba* was more sensitive (lower EC₅₀ values) to atrazine, diuron, irgarol and

pentachlorophenol. In contrast, *L. minor* was more sensitive to Cd, Cu and triclosan. EC₅₀ value differences for the same chemical between these two species were 5-fold or less.

Duckweeds were rarely the most sensitive freshwater plant test species (Table 1). The exceptions were for diquat (*S. punctata*) and Ni (*L. minor*). Duckweeds were in the top five of sensitive species for glyphosate (*L. aequinoctialis*), terbuthylazine (*L. gibba*), linuron (*L. gibba*), metolachlor (*L. gibba*), metribuzin (*L. perpusilla*) and prometryn (*L. gibba*). The EC₅₀ value for another floating species, the fern *Salvinia natans*, was the lowest of the 27 freshwater species exposed to metolachlor.

3.5. Freshwater benthic plants

Benthic macrophytes are useful test species since they lack mobility which increases their vulnerability to chemical exposure. Toxicity information was available for 20 freshwater submersed and emergent species and 14 test chemicals (Table 6). Four species of *Myriophyllum* (water milfoils) were test species and, of these, *M. spicatum* (Eurasian watermilfoil) was used most frequently (10 chemicals). This is not unexpected since *Myriophyllum* spp. have been recommended for use as a test species. A standard test methodology is available and endpoint sensitivities described (Arts et al., 2008; OECD, 2014). Sensitivity of *M. spicatum* has been chemically-specific (Arts et al., 2008; Giddings et al., 2013) which was also observed here. It was not a top 10 sensitive species for any test chemical. *E. canadensis* (common waterweed) was the second most frequently used test species (six chemicals). It was a top 10 sensitive species for three of the six chemicals. It was the most sensitive of all freshwater species to pentachlorophenol (EC₅₀=4 µg L⁻¹). *E. nuttalli* was most sensitive to linuron (EC₅₀=2.5 µg L⁻¹) (Table 1). Four same-chemical comparisons were available for *M. spicatum* and *E. canadensis*. *E. canadensis* was more sensitive to pentachlorophenol, atrazine and Zn. The EC₅₀ values (µg L⁻¹) were 4, 21, and 8,100 for *E. canadensis* and 236, 91, and 20,900 for *M. spicatum* and the same chemicals, respectively. In contrast, *M. spicatum* (EC₅₀=55 µg L⁻¹) was more sensitive than *E. canadensis* (EC₅₀=172.9 µg L⁻¹) to terbuthylazine. The most diverse benthic dataset was for atrazine. EC₅₀ values for the nine test species ranged from 21 to 66,960 µg L⁻¹. Species sensitivities in declining order were: *E. canadensis*>*Ceratophyllum demersum*>*Potamogeton perfoliatus*>*Najas* sp.>*M. spicatum*>*Myriophyllum heterophyllum*>*Hydrilla verticillata*>*Myriophyllum sibiricum*> *Lepidium sativum*.

3.6 Saltwater benthic plants

Toxicity information was available from the ECOTOX database for 10 of the 20 chemicals (Table 6) and 19 saltwater benthic species. Seventeen of the 19 test species were macroalgae or “seaweeds” represented by red (Phylum Rhodophyta, six species), brown (Phylum Ochrophyta, eight species) and green (Phylum Chlorophyta, three species) taxa. Of these, the red seaweed, *Champia parvula*, was the most sensitive of all saltwater test species to Cr (Table 1). The most diverse saltwater database was for Cu. The range of EC₅₀ values (µg L⁻¹) for the 11 test species was 1.4 to 440. Species sensitivities in declining order were: *C. parvula* (red alga)>*Macrocystis pyrifera* (brown) > *Enteromorpha intestinalis* (green)> *Ceramium strictum* (red)> *Lessonia nigrescens* (brown)> *Fucus serratus* (brown)>*Ulva*

pertusa (green)>*Fucus vesiculosus* (brown)>*Gracilaria tenuistipitata* (red)>*Hormosira banksi* (brown)>*Ulva reticulata* (green).

Seagrass meadows are rapidly declining worldwide possibly due to the presence of shoreline chemicals such as herbicides (Wilkinson et al. 2015). Toxicity information for this summary was available for three chemicals and the saltwater *Zostera marina* (common eelgrass) and *Ruppia maritima* (widgeon grass) and the freshwater/brackish water *Vallisneria americana* (tape grass). EC₅₀ values (µg L⁻¹) for atrazine were 60 (*Z. marina*), 330 (*V. americana*) and 10,571 (*R. maritima*). EC₅₀ values (µg L⁻¹) for the anti-foulant, irgarol, were 0.8 (*R. maritima*) and 1.1 (*Z. marina*). The EC₅₀ for diuron and *Z. marina* was 3.2. The sensitivity ranks for seagrass test species relative to other saltwater test species were 9th, 15th and 16th (atrazine), 10th and 14th (irgarol) and 2nd (diuron).

3.7. Sensitivity: Freshwater algae and freshwater floating and benthic macrophytes

EC₅₀ values for the most sensitive freshwater algae were compared to those for the most sensitive duckweed and freshwater benthic species based on information primarily in Tables 1 and 6. EC₅₀ values were lower for algae than duckweeds for 14 of 15 test chemicals (exception, Ni) and less for 11 of 14 comparisons with benthic species (exceptions, linuron, metolachlor and pentachlorophenol). The differences between EC₅₀ values based on these comparisons ranged from 1 to 237-fold.

3.8. Sensitivity: Freshwater and saltwater algae

EC₅₀ values for the most sensitive freshwater algal species were less than values for the most sensitive saltwater taxa for 12 of the possible 15 comparisons (Table 1). The EC₅₀ values for As, Cr and Pb were less for saltwater taxa. The differences between EC₅₀ values ranged from 1 to 80-fold. A more specific freshwater-saltwater comparison is shown in Table 2 for the freshwater green microalga, *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*, *Selenastrum capricornutum*) and the saltwater diatom, *S. costatum*. These are commonly used test species in phytotoxicity tests due to the availability of culture techniques and standardized test procedures from standard writing organizations and regulatory agencies (examples, OECD, 2006; USEPA, 2012; ASTM, 2012). The results of the 17 possible across-species comparisons show an almost equal sensitivity split based on EC₅₀ values (Table 2). EC₅₀ values for *R. subcapitata* were less than those for *S. costatum* for nine chemicals and greater for eight chemicals. The differences for EC₅₀ values between the two species and same chemical ranged between 1 and 45-fold but were usually less than 10 (exceptions, As, diquat and triclosan).

3.9. Sensitivity: Duckweeds and benthic species

Duckweed species are assumed by some to be suitable surrogates for other freshwater macrophytes in the risk assessment process (Giddings et al., 2013). This ability was chemical-specific based on a comparison of EC₅₀ values for 14 test chemicals (Table 6). EC₅₀ values for duckweeds and Cd, Cu, diquat, Pb, terbutylazine and Zn were less than those for freshwater benthic macrophytes (EC₅₀ difference=2-137-fold). Duckweeds were less sensitive (greater EC₅₀ values) than benthic species for the remaining eight chemicals.

EC₅₀ values between duckweed and benthic macrophytes for this latter comparison differed 2-fold or less except for pentachlorophenol and linuron.

The chemical-specific trend observed above for the duckweed-freshwater benthic species comparisons is similar to that when the duckweed surrogate assumption is extended to represent results for saltwater species (Table 6). The results for the 10 possible same-chemical comparisons indicate duckweed EC₅₀ values were less than those for saltwater benthic species to atrazine, Ni, pentachlorophenol and Zn but with the exception of Ni, not by much. Conversely, EC₅₀ values were greater for duckweeds and Cd, Cr, Cu, Pb, irgarol and diuron; differences between EC₅₀ values ranged from 2 to 59-fold.

Very few literature sources are available that report duckweed-benthic plant species sensitivity differences to the same chemicals. Giddings et al. (2013) reported that *L. gibba* was among the most sensitive species to 50% of 14 unnamed herbicides and fungicides and *M. spicatum* was among the most sensitive to 25% of the same chemicals. Results of this summary (Table 6) show that *L. gibba* was the most sensitive duckweed species to 6 of 15 (40%) chemicals and *M. spicatum* was the most sensitive benthic species to 7 of 14 (50%) chemicals. One same-toxicant comparison is available for these species. EC₅₀ values (µg L⁻¹) for terbuthylazine were 16 (*L. gibba*) and 55 (*M. spicatum*).

4.0. Sensitivity: Freshwater and saltwater benthic species

EC₅₀ values for freshwater and saltwater benthic species exposed to the same nine test chemicals are compared in Table 6. Saltwater species were more sensitive (lower EC₅₀ values) to all chemicals but atrazine and pentachlorophenol. The EC₅₀ values for freshwater and saltwater benthic species were almost equivalent for diuron and the anti-foulant algicide, irgarol, whereas EC₅₀ values for the remaining test chemicals and benthic species differed approximately 3- to 352-fold.

4. Species-sensitivity distribution plots and final plant values

Examples of the SSD plots used to assess the ability of the five species data set to serve as a surrogate for the all test species data sets appear in Figure 2. Each of the four graphs consists of one plot based on EC₅₀ values for all test species and one plot based on EC₅₀ values for the five FIFRA-recommended species. HC₅ values, and 95% confidence limits appear in Table 3 for the two data sets and each test chemical.

Differences between HC₅ values for same test chemical and the all species and five species data sets were usually within a 4-fold range except for Zn (Table 3; Fig. 3). HC₅ values (µg L⁻¹) ranged from 0.07 (irgarol) to 2,189.4 (glyphosate) and 0.04 (irgarol) to 7,979.8 (glyphosate) for all test species and five test species data sets, respectively. HC₅ values for the five species data sets were less than those for the all test species data sets and 12 chemicals and greater for seven chemicals. The largest difference was for Zn (78-fold) where the EC₅₀ value based on the all test species data set greatly overestimated the EC₅₀ for the five species data, Zinc is an outlier because of the discrepancy between the proportionality ranks for FIFRA species in the FIFRA and all species data bases. See Thursby and Lewis (2015) for more detail. In contrast to Zn, the HC₅ values for Cr were

identical. The differences between values were not affected by the relative contribution (%) of results) of the FIFRA species to the all test species data sets used in the comparisons (data not shown). The results of the HC₅ comparisons suggests that the five test species could serve as an interim plant MDR for aquatic life criteria development with the knowledge that their use would err more commonly on the conservative side providing a degree of environmental safety.

The Plant Final Value (FPV) used for water quality criteria development in the U.S. is based on the lowest toxicity result with an aquatic plant (alga or macrophyte) (Stephan et al., 1985). To provide some insight on the value of this approach, the lowest EC₅₀ value for the five test species was compared to the SSD-generated HC₅ value for the same chemical (Table 3). The HC₅ values based on the cumulative response of all test species were less than the lowest EC₅₀ value for 16 of 20 test chemicals. Differences between EC₅₀-HC₅ values were usually less than 10-fold (range= 1 to 32) for the 16 chemicals. The HC₅ and EC₅₀ values were virtually equivalent for Pb, metribuzin, prometryn and terbuthylazine. The greater EC₅₀ values suggest that the use of the five species as the source of the FPV would be a less conservative approach than using all test species-based HC₅ values, at least for the test chemicals and test species evaluated here.

5. Discussion and conclusions

Toxicity information for aquatic faunal species will continue as the cornerstone for most regulatory chemical risk decisions and water quality criteria determinations due to a relatively large and diverse toxicity data base and supporting knowledge of sensitive taxa. Understanding the toxicities of chemicals to aquatic plants and their consideration in environmental safety decisions have been slow to advance with limited evidence of this trend changing soon due to the ongoing assumption of their relative insensitivity and to the limited toxicity information available for all but a few chemicals and test species. For this pattern to change, additional across-species toxicity information (fauna-flora; flora-flora) is needed that includes non-standard test species, endpoints and life stages as encouraged by Ruden et al., (2016) and Buchwalter et al., (2017) to support interspecies correlation estimation models (Brill et al. 2016; He et al. 2017). These advancements should also include some consideration for greater diversity of test chemicals (non-herbicides), mixture toxicity (Magnusson et al., 2010; Wilkinson et al., 2015) and increased focus for phytocidal concentrations needed to compare aquatic fauna-flora acute toxicities and to establish plant acute/chronic toxic effect ratios.

The toxicity databases for algae and more advanced aquatic vascular species are uneven. Considerably more information is available for freshwater and saltwater algae which has served as the foundation of the phytotoxicity database due largely to the 40 yr availability of standardized algal toxicity tests. This algal-centric dominance represents a possible bias concerning conclusions for interspecific plant sensitivities to anthropogenic chemicals as recognized 34 years ago (Fletcher, 1990). This uncertainty should decrease in the future as toxicity information and test methods become more available for other species, such as that occurring for freshwater macrophytes (Brain et al., 2006; Arts et al., 2008; Maltby et al., 2010; Giddings et al., 2013; OECD, 2014). The diversity of the saltwater phytotoxicity

database is also increasing, although slowly, in reaction to the possible role of shoreline chemicals for the worldwide coverage declines of near-coastal vegetated ecosystems. Toxic effect concentrations for herbicides, oil and other chemicals are becoming more available for mangroves, seagrass, macroalgae; coral algal symbionts and benthic microalgae (Eklund and Kautsky, 2003; Jones, 2005; Lewis and Devereaux, 2009; Magnusson et al., 2010; Lewis et al., 2011; Larras et al., 2012; Bayen, 2012; Lewis and Pryor, 2013; Wilkinson et al., 2015). These efforts are supported by relatively recent test methods reported by standard writing organizations for marine diatoms, macroalgae, and coastal wetland plants (ASTM, 2011; American Public Health Association et al., 2012; ISO, 2017). The challenge to the regulatory community and risk assessors will be to utilize the expanding freshwater and saltwater databases for future chemical safety decisions and determinations for numerical benchmarks for water and sediment quality, wetlands (USEPA 1990), seagrass (Nelson, 2009) and contaminants of emerging concern (USEPA 2008). Modernization of water quality criteria has been an ongoing objective in the U.S. since 1994 and efforts continue to attain this goal (Russo 2002; USEPA Science Advisory Panel, 2005; USEPA, 2015; DeForest et al., 2017; Buchwalter et al., 2017).

The results for the many species comparisons of EC_{50} values made here were multidirectional, reflecting the often species-specific responses to the same test chemical. This was observed for algae, duckweed and benthic macrophytes as well as for freshwater and saltwater species. Freshwater algae were often more sensitive (lower EC_{50} values) than saltwater algae but not for the two more commonly recommended test species for which the current phytotoxicity database is largely based. EC_{50} values for duckweeds were more equivalent to those for freshwater macrophytes than for saltwater species and saltwater benthic species were typically more sensitive than freshwater benthic taxa. The multidirectional results of these and other sensitivity differences indicates that a requirement for a standard number of aquatic plant test species representing a standard number of taxonomic families, as done for faunal species (Stephan et al., 1985), would be premature for the aquatic life criteria process in the U.S.

In contrast to the use of specific species and taxonomic groups, when the focus is on the representativeness of the distribution of EC_{50} values, five microalgal-dominated species provided an often conservative approximation of HC_5 values based on larger and more species-diverse toxicity data sets. This collective ability to reasonably represent the response of the wide spectrum of the aquatic plant community, at least that defined by a subset of results from the ECOTOX database, suggests their suitability as an interim MDR for development of aquatic life criteria. The value of the five species is less obvious, however, to provide the Final Plant Value in the same process since the lowest of their EC_{50} values was usually greater than the HC_5 value for the same chemical. It is noteworthy also that this summary provides support for the current use of the five FIFRA test species to screen pesticide phytotoxicities. The lowest EC_{50} value for the five species was one of the three lower for 16 of the 20 test chemicals. This relative sensitivity parallels that reported elsewhere for similar chemicals (Vervliet-Scheerbaum et al. 2006; Giddings et al., 2013). Finally, the conclusions of this summary are specific to our approach which was based on EC_{50} values for predominately heavy metals and herbicides, growth-related response parameters, combined toxicity results for freshwater and saltwater non-vascular and vascular

test species, log-normal distributions and HC₅ values. Conclusions based on results for other test chemicals, test species, response parameters and calculations (mean or median EC₅₀ values) may differ.

Acknowledgments

Database searches and quality assurance checks were performed by personnel from the Great Lakes Environmental Center (Traverse City, MI), under contract to U.S. Environmental Protection Agency's Office of Water, Washington D.C. Paul Soderlind (CSRA Inc., Falls Church, VA) provided graphics support.

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Raphidocelis subcapitata
(Green microalga)

Microcystis aeruginosa
(Cyanobacterium)

Skeletonema costatum
(Centric diatom)

Lemna gibba
(Duckweed)

Myriophyllum spicatum
(Eurasian watermilfoil)

Macrocystis pyrifera
(Giant kelp)

Vallisneria americana
(Tape grass)

Typha latifolia
(Cattail)

Spartina alterniflora
(Saltmarsh cordgrass)

Figure 1.

Examples of freshwater and saltwater test species included in the sensitivity comparisons and SSD plots. Photos from Encyclopedia of Life include *Microcystis aeruginosa* (William Bourland), *Lemna gibba* (Biopix), *Typha latifolia* (Hans Hillewaert), *Macrocystis pyrifera* (Claire Fackler, NOAA), *Spartina alterniflora* and *Vallisneria americana* (USDA NRCS Plant Database). Photo for *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*, *Selenastrum capricornutum*) used with permission from John Kinross (algalweb). *Myriophyllum spicatum* from National Park Service and *Skeletonema costatum* from NOAA/Sea Grant. Photographs not to scale.

Figure 2.

Examples of log normal probability plots for species sensitivity (Equation 3) and four test chemicals spanning the range of available toxicity information. Open markers are for the all test species data set and closed markers are for the five FIFRA-recommended species. Solid and dashed lines are the regression best fit for each data set and triangles represent $> \text{EC}_{50}$ values. Vertical dashed line intersects the regressions at the log of the HC_5 value. N-number of test species/toxicity results

Figure 3.

Relationship between the 5th percentile hazard concentrations (HC₅) for the all test species data sets and the FIFRA-recommended five-species data sets. Solid black line is the one-to-one relationship. FIFRA species-based HC₅ values above line are less than the all test species data set HC₅ values. HC₅ values below line are greater than HC₅ values based on all available tests species. Values within the two dashed lines are within a factor of four of a one-to-one relationship.

Table 1

Twenty test chemicals that met the ten chemical minimum data requirement. Most sensitive test species and corresponding EC₅₀ values (µg L⁻¹) are shown for each test chemical. Test species in bold are FIFRA-recommended. G-green microalga; BG-cyanobacteria (blue-green microalgae); DW-duckweed; D-diatom; BM-benthic macrophyte; FF-floating fern; GF-green flagellate; RA- attached red alga. N=number of total test species.

Test Chemicals	N	Freshwater		Saltwater	
		EC ₅₀	Species	EC ₅₀	Species
Arsenic V	11	159.3	<i>Scenedesmus acutus</i> (G)	2	<i>Isochrysis galbana</i> (G)
Atrazine	69	13.0	<i>Pseudanabaena galeata</i> (BG)	17.2	<i>Amphidinium operculatum</i> (GF)
Cadmium	42	10.8	<i>Scenedesmus acutus</i> (G)	60	<i>Chaetoceros calcitrans</i> (D)
Chromium VI	34	104	<i>Chlorella protothecoides</i> (G)	42.4	<i>Champia parvula</i> (RA)
Copper	76	<0.75	<i>Microcystis aeruginosa</i> (BG)	0.6	<i>Bellerophonella polymorpha</i> (D)
Diquat	13	0.75	<i>Spirodella punctata</i> (DW)	>2,940	<i>Skeletonema costatum</i> (D) [/]
Diuron	46	1.3	<i>Chlorella pyrenoidosa</i> (G)	2.3	<i>Coccolithus huxleyi</i> (D)
Glyphosate	11	3,530	<i>Chlorella pyrenoidosa</i> (G)	12,000	<i>Skeletonema costatum</i> (D) [/]
Irgarol	48	0.013	<i>Ulmaria ulna</i> (D)	0.1	<i>Tetraselmis</i> sp. (G)
Lead	19	990	<i>Anabaena flos-aquae</i> (BG)	19.5	<i>Skeletonema costatum</i> (D)
Linuron	10	2.5	<i>Elodea nuttalli</i> (BM)	35.9	<i>Skeletonema costatum</i> (D) [/]
Metolachlor	28	50	<i>Salvinia natans</i> (FF)	61	<i>Skeletonema costatum</i> (D) [/]
Metribuzin	18	8.1	<i>Raphidocelis subcapitata</i> (G)	8.8	<i>Skeletonema costatum</i> (D) [/]
Nickel	13	191	<i>Lemna minor</i> (DW)	150	<i>Chlorella vulgaris</i> (G)
Pentachlorophenol	27	4	<i>Elodea canadensis</i> (BM)	35.3	<i>Skeletonema costatum</i> (D) [/]
Prometryn	13	1	<i>Navicula pelliculosa</i> (D)	53	<i>Dunaliella tertiolecta</i> (GF)
Selenium	13	89	<i>Scenedesmus acutus</i> (G)	7,664	<i>Skeletonema costatum</i> (D) [/]
Terbutylazine	15	7.9	<i>Raphidocelis subcapitata</i> (G)	31	<i>Skeletonema costatum</i> (D) [/]
Triclosan	20	1.0	<i>Scenedesmus subspicatus</i> (G)	3.6	<i>Dunaliella tertiolecta</i> (GF)
Zinc	28	4.1	<i>Synechococcus leopoliensis</i> (BG)	58.9	<i>Asterionella japonica</i> (D)

[/] only saltwater test species evaluated

Table 2

Number of freshwater (FW) and saltwater (SW) test species and ranges of EC₅₀ values (µg L⁻¹) for each test chemical. EC₅₀ values also compared for the same test chemical and the freshwater green microalga, *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum*, *Pseudokirchneriella subcapitata*) and the marine centric diatom, *Skeletonema costatum*. ND-no data

Test Chemicals	Number of Test Species		EC ₅₀ (range)		<i>Raphidocelis subcapitata</i>	<i>Skeletonema costatum</i>
	FW	SW	FW	SW		
Arsenic V	2	8	159 >2,400	2 >100	228	11.7
Atrazine	53	16	13–78,799	17.2–10,571	73.2	35.7
Cadmium	26	16	10.8–8,350	60–48,900	43.1	178.1
Chromium VI	28	9	104–270,680	42.4–41,378	122.2	1,200
Copper	32	44	<0.75–11,925	0.6–12,740	14.8	114.2
Diquat	12	1	0.75–2,940	>2,940	66	>2,940
Diuron	34	12	1.3–2,606	2.3–6,820	7.1	5.9
Glyphosate	11	1	3,530–68,000	12,000	9,513	12,000
Irgarol	29	19	0.013–>253	0.1–7.7	3.2	0.41
Lead	6	12	990–363,000	19.5–46,700	ND	19.5
Linuron	9	1	2.5–80	35.9	67	35.9
Metolachlor	27	1	50–310,151	61	86.9	61
Metribuzin	17	1	8.1–2,953	8.8	8.1	8.8
Nickel	7	6	191–446,044	150–17,000	263.6	ND
Pentachlorophenol	21	6	4–34,300	35.3–3,000	147.8	35.3
Prometryn	11	2	1–85	7.6, 53	12	7.6
Selenium	12	1	89–112,250	7,664	820	7,664
Terbutylazine	14	1	7.9–254	31	7.9	31
Triclosan	8	2	1.0–620	3.6, >66	2.2	>66
Zinc	23	11	4.1–75,852	58.9–7,100	52.9	ND

Table 3

Parameters for linear regressions based on toxicity results for all test species and the five FIFRA-recommended test species. Lowest EC₅₀ values (µg L⁻¹) for the FIFRA test species shown for Final Plant Value analysis (Stephan et al. 1985). HC₅=hazard concentration fifth percentile (µg L⁻¹); CL=confidence limit (µg L⁻¹).

Test Chemicals	All Test Species					Five Test Species					
	Slope	Intercept (ln EC ₅₀)	r ²	HC ₅	95% CL	Slope	Intercept (ln EC ₅₀)	r ²	HC ₅	95% CL	Lowest EC ₅₀
Arsenic V	2.364	4.273	0.965	1.5	0.8	2.957	5.023	0.958	1.2	0.2	11.7
Atrazine	1.022	4.466	0.937	16.2	15.1	0.987	4.165	0.892	12.7	4.1	31.5
Cadmium	2.336	6.450	0.979	13.6	11.0	1.674	4.770	0.948	7.5	2.1	31
Chromium VI	1.503	6.677	0.949	67	54.8	1.224	6.217	0.906	67	11.8	122
Copper	2.436	4.345	0.984	1.4	1.2	1.598	4.276	0.952	5.2	1.6	14.8
Diquat	2.824	4.134	0.908	0.6	0.2	3.086	4.049	0.893	0.36	0.01	19
Diuron	1.256	2.720	0.944	1.9	1.8	0.749	2.464	0.881	3.4	1.9	5.9
Glyphosate	0.932	9.224	0.895	2,189.4	1,409	0.293	9.467	0.972	7,979.8	6,783.9	9,513
Irgarol	1.911	0.470	0.939	0.07	0.06	1.756	-0.316	0.971	0.04	0.01	0.1
Lead	2.837	7.665	0.985	20.0	14.4	3.122	7.013	0.864	6.5	0.1	19.5
Linuron	1.198	3.196	0.901	3.4	1.9	0.752	3.474	0.946	9.4	5.2	13.7
Metolachlor	2.429	7.424	0.897	30.8	14.5	1.568	5.280	0.918	14.9	3.4	61
Metribuzin	0.667	3.130	0.966	7.6	6.8	1.414	2.930	0.736	1.8	0.1	8.1
Nickel	2.632	8.092	0.939	43.1	18.9	1.964	6.667	0.842	31.1	1.9	264
Pentachlorophenol	1.956	5.721	0.901	12.2	7.9	1.086	4.597	0.955	16.6	7.7	35.6
Prometryn	1.482	2.662	0.911	1.3	0.7	1.680	2.135	0.876	0.5	0.1	1
Selenium	2.341	8.212	0.957	78.4	42.9	1.686	8.462	0.978	295.6	130.6	820
Terbutylazine	1.298	4.107	0.941	7.2	5.0	1.291	3.053	0.932	2.5	0.8	7.9
Triclosan	2.706	2.908	0.961	0.2	0.1	2.335	2.441	0.905	0.25	0.02	1.3
Zinc	2.762	7.467	0.965	18.6	12.5	4.090	5.291	0.942	0.24	0.01	52.9

Table 4

Ranges of EC₅₀ values ($\mu\text{g L}^{-1}$) for different test species within the same Phyla Chlorophyta (green algae), Cyanophyta (cyanobacteria/blue-green algae) and Chrysophyta (diatoms). Information presented for two or more test species. Number of test species in parenthesis.

Test Chemicals	Freshwater			Saltwater
	Chlorophyta	Cyanophyta	Chrysophyta	Chrysophyta
Arsenic				12->100 (4)
Atrazine	15-19,852 (4)	13-5,369 (8)	60-56,000 (14)	36-61 (3)
Cadmium	11-9,700 (11)	120; 18,350 (2)	31-9,300 (6)	60-12,362 (6)
Copper	4-8,961 (15)	< 0.8-29 (3)	125-10,429 (5)	0.6-7,700 (9)
Diuron	1-559 (9)	23-490 (4)	4-2,606 (14)	4-36 (4)
Irgarol	0.5-3.2 (5)		0.01->253 (16)	0.3-1.1 (4)
Lead	1,050-63,800 (3)			20-10,960 (6)
Metolachlor	87-12,717 (5)	1,200->5,000(3)	220-310,151 (12)	
Pentachlorophenol	80-34,300 (9)	50; 130 (2)		35-3,000 (3)
Zinc	34-75,852 (9)		2,524; 10,100 (2)	59-37,100 (5)

Table 5

EC₅₀ values ($\mu\text{g L}^{-1}$) available for duckweed test species and the 20 test chemicals.

Test Chemicals	<i>Lemna aquinoctialis</i>	<i>Lemna gibba</i>	<i>Lemna minor</i>	<i>Lemna perpusilla</i>	<i>Lemna trisulca</i>	<i>Spirodella punctata</i>	<i>Spirodella polyrrhiza</i>	<i>Wolffia globosa</i>
Arsenic V			630					
Atrazine	69.1	31.5	60					
Cadmium		800	191.1				54.1	500
Chromium VI			990.3					1,700
Copper	943.4	314.4	82.6				3,300	
Diquat			4			0.75		
Diuron	9.3	15.7	25.0	15			41	
Glyphosate	3,889	12,400						
Irgarol		1.6	8.1					
Lead							7,600	
Linuron		25						
Metolachlor		120.8						
Metribuzin	44.9	160		16				
Nickel			191				4,500	
Pentachlorophenol		250	1,250		1,282			
Prometryn	41	11.8		13			85	
Selenium			2,903					
Terbutylazine		16			254		182.4	
Triclosan		57.1	26.3					
Zinc			9,600				48,600	

Table 6

EC₅₀ values ($\mu\text{g L}^{-1}$) for the most sensitive species of floating duckweed and freshwater and saltwater benthic macrophytes exposed to the same chemical. ND- no data.

Test Chemicals	Duckweeds		Freshwater Benthic Taxa		Saltwater Benthic Taxa	
	Species	EC ₅₀	Species	EC ₅₀	Species	EC ₅₀
Atrazine	<i>Lemna gibba</i>	31.5	<i>Elodea canadensis</i>	21	<i>Zostera marina</i>	60
Cadmium	<i>Spirodela polyrrhiza</i>	54.1	<i>Myriophyllum spicatum</i>	7,400	<i>Laminaria saccharina</i>	21
Chromium	<i>Lemna minor</i>	990.3	<i>Myriophyllum spicatum</i>	915.8	<i>Champia parvula</i>	42.4
Copper	<i>Lemna minor</i>	82.6	<i>Myriophyllum spicatum</i>	250	<i>Champia parvula</i>	1.4
Diquat	<i>Lemna minor</i>	4	<i>Myriophyllum sibiricum</i>	84.7	ND	
Diuron	<i>Lemna aequinoctialis</i>	9.3	<i>Myriophyllum spicatum</i>	5	<i>Zostera marina</i>	3.2
Irgarol	<i>Lemna gibba</i>	1.6	<i>Myriophyllum verticillatum</i>	1.1	<i>Ruppia maritima</i>	0.8
Lead	<i>Spirodela polyrrhiza</i>	7,600	<i>Myriophyllum spicatum</i>	363,000	<i>Gracilaria tenuistipitata</i>	4,000
Linuron	<i>Lemna gibba</i>	25	<i>Elodea nuttalli</i>	2.5	ND	
Metolachlor	<i>Lemna gibba</i>	120.8	<i>Ceratophyllum demersum</i>	70	ND	
Metribuzin	<i>Lemna perpusilla</i>	16	<i>Ceratophyllum demersum</i>	14	ND	
Nickel	<i>Lemna minor</i>	191	ND		<i>Macrocystis pyrifera</i>	2,000
Pentachlorophenol	<i>Lemna gibba</i>	250	<i>Elodea canadensis</i>	4	<i>Macrocystis pyrifera</i>	300
Terbutylazine	<i>Lemna gibba</i>	16	<i>Myriophyllum spicatum</i>	55	ND	
Zinc	<i>Lemna minor</i>	9,600	<i>Myriophyllum spicatum</i>	20,900	<i>Macrocystis pyrifera</i>	10,000