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New Technique for Estimating Thresholds of Toxicity in Ecological Risk Assessment

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The use and utility of the no observed effect concentration (NOEC) in ecological risk assessment is a contentious issue. One concern is that the NOEC is not representative of a concentration at which no biologically significant effect is occurring. A new method has been developed to estimate the threshold of toxicity, or a true NOEC, for aquatic plants. The method involves determining the effective concentration (EC_x) of a number of endpoints from one species. These EC_x values are plotted on a log-probability scale. The *x*-intercept, or a low centile, of the distribution can be interpreted as the threshold of toxicity for that plant at that response level. This threshold is the concentration at which no effects should be observed for any endpoint above that response level. It is based on the assumptions that multiple effect measures from a single species will be log-normally distributed and that the distribution contains all possible endpoints for that species. The thresholds and the distributions can then be used as a substitute for the NOEC or EC_x in risk assessment techniques, such as hazard quotients and probabilistic ecological risk assessment. This new method of estimating toxicity thresholds is more realistic than the use of arbitrary uncertainty factors, is more conservative than current probabilistic risk assessment methods, allows for simple comparison between species and exposure duration to a toxicant, and may be useful for assessing mixture toxicity. This technique was applied to field derived data with *Lemna gibba*, *Myriophyllum spicatum*, and *M. sibiricum* to assess potential risks from monochloroacetic acid (MCA). Using this new risk assessment method, we conclude that MCA does not appear to pose a risk to aquatic macrophytes under field conditions at current environmental concentrations.

Introduction

The use of nontarget plants in the regulatory risk assessment of pesticides in both Canada and the United States has recently come under review with recommendations for increasing the number of species used in the process (*1*). The rooted macrophyte *Myriophyllum sibiricum* has been suggested as a new possible mandatory test species for the registration of pesticides. Toxicity testing with *Myriophyllum* spp. can evaluate numerous effect measures (*2*). These data can then be used to conduct a risk assessment for that compound. Current risk assessments tend to rely on the deterministic approach of hazard quotients (HQ), by which an effect concentration or a statistically derived no observed effect concentration (NOEC) is divided by an exposure concentration to determine if an effect might be expected (*3*). Worst-case exposure and effect data are generally used in this type of assessment. More complex estimates of risk from exposure to contaminants for aquatic communities involve the use of probabilistic ecological risk assessments (*4*, *5*). These methods usually rely on EC_{50} or LC_{50} estimates and occasionally NOEC values generated under laboratory conditions for multiple species to create species sensitivity distributions. Typically, a low centile of the toxicity distribution of these laboratory-derived values for the compound of interest is selected as a level to protect or below which impacts may occur but are deemed acceptable (*4*−*7*). The underlying assumption is that impacting a small proportion of the species will not result in irreparable harm to the aquatic community due to functional redundancy within the ecosystem (*4*, *5*). Other measures of effect such as the EC_{10} would be more protective of ecosystem structure and function than the LC_{50} or EC_{50} but are seldom found in toxicity databases or the published literature. Concentrations that do not cause direct mortality in an organism may affect other endpoints, such as reproduction or photosynthetic rate, and have impacts not only on the structure of an ecosystem but also on its function. The general lack of inclusion of measures of chronic effects, endpoints other than mortality, and the tolerance of some structural effects in probabilistic ecological risk assessment (PERA) has drawn criticism, especially when dealing with ecosystems or species deemed to be at risk and negative public perception of the "acceptable" loss of a proportion of species in any given environment (*8*−*11*). As well, the apparent use of PERA to justify the entry of toxic compounds into aquatic environments has been a concern (*12*).

Both deterministic and probabilistic risk assessments rely on accurate and reliable data to be conducted properly. The use of NOECs, the highest test concentration that is not statistical different ($p > 0.05$) from control values, in ecological risk assessment has been criticized for a variety of reasons. These concerns include the following: (i) the NOEC must be one of the concentrations tested, (ii) the NOEC tends to increase as the precision of the study decreases, and (iii) the NOEC depends on the chosen significance level (*13*−*16*). Studies with aquatic plants that report both an EC_x value and a NOEC often show the that NOEC is well within the range of the estimated EC_{50} (*17*). Clearly, a new means of estimating a NOEC or threshold of toxicity is required. Suggestions have been made that the EC_5 or EC_{10} (*14*, *15*, *18*) or the estimation of no effect concentrations using threshold models (*16*) for individual endpoints should replace the NOEC. While each technique is valid for dealing with individual endpoints, a number of problems arise. If, for example, dealing with species such as aquatic plants, which endpoint(s) do you evaluate? How does the risk assessor know that the most sensitive endpoint has been observed? Indeed, a wide range in endpoint sensitivity has been observed with *Myriophyllum* spp. in toxicity tests (*19*−*25*). Also, the plant tests generate estimates of effects for numerous endpoints that would not be put to full use in either a HQ or a PERA approach.

Since many distributions appear to be log-normally distributed (*26*), it is reasonable to assume that the distribution of endpoint sensitivities within an organism might be as well. The rationale is that, within an organism, there are a finite number of effect measures that could be evaluated with some being more sensitive than others. These intraspecies toxicity values themselves may be log-normally distributed, lending themselves to use in a modified PERA. This

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results in a distribution of effect measures that pertain to a single species. Since the distribution no longer includes only mortality but other effect measures that will not be as detrimental as death of a species on the ecosystem, the risk assessment becomes more protective. The distribution can be used to estimate a more realistic threshold of response than is possible with arbitrary uncertainty factors applied to EC_x estimates or currently calculated NOECs.

The data used in this risk assessment was derived from a field study with *Myriophyllum spicatum*, *M. sibiricum*, and *Lemna gibba* on the toxicity of the haloacetic acid (HAA) monochloroacetic acid (MCA) (*27*). HAAs are environmental contaminants that have been detected in aquatic ecosystems, rainwater, fog, and snow (*28*−*32*). MCA is commonly detected in aquatic systems around the world (*30*, *31*) and has been demonstrated to be phytotoxic (*33*), especially to aquatic plants such as algae. Effects have been documented at concentrations as low as 25 μg/L (72 h biomass EC_{50}) for the green algae *Scenedesmus subspicatus* (*34*). MCA has a number of sources including water disinfection via chlorination (*35*) and as the degradation byproduct of herbicides (*36*). Moreover, MCA is produced at a rate of 300 000 t annually as an intermediate in the production of other chemicals, including some herbicides (*37*). Therefore, aquatic organisms are potentially at risk from MCA exposure. While there is some information on toxicity of MCA toward unicellular plants, information is scarce on potential effects on organisms such as the macrophytes *Myriophyllum* spp. and *L. gibba*, which can form the bulk of the standing biomass in aquatic communities (*38*, *39*).

The purpose of this study was to (i) determine the validity of plotting distributions of effect measures from single species to estimate a threshold of toxicity and (ii) conduct an environmental risk assessment for MCA using both HQ and probabilistic techniques with data derived from these distributions and to compare and contrast the results with more common approaches. Recommendations and other potential applications, such as mixture assessment, of the technique are discussed.

Experimental Section

Effect Measure Distributions. The plotting of effect measure distributions followed that of Solomon and Giesy (*6*) with some changes outlined below. Toxicity data were obtained from a microcosm field study examining the effects of MCA on three common aquatic macrophytes; *Myriophyllum spicatum* L. (Haloragaceae), *M. sibiricum* Komarov (Haloragaceae), and *Lemna gibba* G3 (*27*). Toxicity was assessed at four time points (days 4, 7, 14, and 28) for *Myriophyllum* spp. and at one time point for *L. gibba* (day 21). End points monitored for *Myriophyllum* spp. were plant length, total biomass, root number, root length, longest root length, wet and dry mass, node number, chlorophyll *a*, chlorophyll *b*, and carotenoid content. End points monitored for *L. gibba* were frond number, plant number, wet and dry mass, frond and plant growth rate, chlorophyll *a*, and chlorophyll *b*. Concentration−response relationships were modeled using nonlinear regression techniques (*40*). Effect measures were calculated at EC_{10}, EC_{25}, and EC_{50}.

Effect measures at each of the three calculated levels (EC_{10}, EC_{25}, and EC_{50}) for each of the four dates evaluated (4, 7, 14, and 28 days) were plotted as a cumulative frequency distribution using a probability scale on the *y*-axis as a function of the log concentration (*4*). This was done with *L. gibba* for a 21 day exposure duration to MCA. Plotting positions were expressed as percentages and calculated from the Weibull formula:

$$100 \times i/(n + 1) \quad (1)$$

where *i* is the rank of the datum and *n* is the total number of data points in the data set (*41*). Data were plotted, and linear regressions on the transformed data were calculated using SigmaPlot 5 (Jandel, San Rafael, CA). In this way, an effect measure distribution for *M. spicatum* at the EC_{10} level for day 4 was plotted and so forth. Pigment data was not used for *Myriophyllum* spp. as no concentration−response relationship was evident with the concentrations used. Plotting positions were also calculated using the Blom equation (eq 2), which has been observed to be better suited for smaller data sets (low *n*) such as these (*42*). The Blom equation is

$$100 \times (i - 0.375)/(n + 0.25) \quad (2)$$

where *i* is the rank of the datum and *n* is the total number of data points in the data set. Using the Blom equation, less extrapolation is required beyond the highest and lowest values in the distribution.

We plotted distributions at three effect levels. The distributions of the EC_{10} were plotted as this measure has been suggested as a more accurate estimation of no effect concentration than the statistically derived NOEC (*14*, *15*). The distributions of the EC_{25} were plotted as this has been described as the threshold for ecological relevance for changes in plant biometrics (*43*). Finally, the EC_{50} distributions were also plotted as this is the effect measure commonly used in traditional probabilistic risk assessments. Toxicity data for the two *Myriophyllum* spp. were also combined to construct effect measure distributions for this assemblage of species at specific effect levels and times. This combined distribution is assumed to represent the genus *Myriophyllum*. The log-transformed effect measure values were tested for normality as recommended by Burmaster and Hull (*26*) following the method outlined in Gilbert (*44*) using the *W*-test.

Calculation of a Toxicity Threshold. A low centile from a distributions of EC_{10} values could be interpreted as a threshold of toxicity, or a "true" NOEC, that would be protective of the entire plant or organism from any impact on an endpoint. The use of the EC_{10} as a substitute for the statistical NOEC as a measure of low toxicity has been recommended (*13*−*15*, *46*). Since a log-normal cumulative frequency distribution does not contain a zero *y* value due to the probability scale, a low centile of 0.1% was chosen. The concentration of MCA at this centile was calculated from the effect measure distribution and deemed to be the threshold of toxicity for that specific distribution. This is a level of impact that would be highly protective of the plant while still being an acceptable extrapolation of the data. Studies have utilized the 1st centile as it was felt this could not be statistically distinguished from 0% (*47*). Others have advocated the use of 0.01% as a level of negligible effect (*48*, *49*) as it is the equivalent to the probit value of 99.99 used for 100% mortality. The regression equations for the distributions are provided so that other levels of effect can be calculated (Tables 1 and 2).

Probabilistic Ecological Risk Assessment. To conduct a probabilistic ecological risk assessment, both toxicity data and contaminant exposure data are required. Exposure data for MCA were derived from Health Canada (*45*) for water treatment plants, from Scott et al. (*31*) for Canadian lakes, and from Berg et al. (*30*) for Swiss rivers. Data reported as below the limit of detection were included in the ranking as zero values but not in the plotting of the cumulative frequency distribution. The procedure assumes that these values are distributed in a continuum of the actual detected values (*4*).

The probability of exceeding the estimated thresholds of toxicity was calculated using joint probability curves, derived from the exposure and effect measure distributions, using the method outlined in Solomon and Giesy (*6*) and is readily

TABLE 1. Regression Coefficients and Intercepts for Monochloroacetic Acid Field Toxicity Distributions for *Myriophyllum spicatum*, *M. sibiricum*, and *Lemna gibba* As Calculated Using the Weibull Equation

distributions	$y = ax + b$[a]			regression intercepts (mg/L)[b]			
	a	*b*	r^2	toxicity threshold	10th centile	*n*[c]	*W*-test ($p > 0.05$)
M. spicatum day 4 EC_{10}	1.70	−0.60	0.95	0.03	0.40	7	pass
M. spicatum day 7 EC_{10}	2.34	−0.85	0.94	0.11	0.65	7	pass
M. spicatum day 14 EC_{10}	1.81	−0.19	0.94	0.02	0.25	7	pass
M. spicatum day 28 EC_{10}	3.18	−0.90	0.97	0.20	0.76	7	pass
M. spicatum day 4 EC_{25}	2.53	−1.78	0.96	0.30	1.57	7	pass
M. spicatum day 7 EC_{25}	3.39	−2.37	0.96	0.61	2.09	7	pass
M. spicatum day 14 EC_{25}	3.72	−1.66	0.94	0.41	1.26	7	pass
M. spicatum day 28 EC_{25}	4.50	−2.50	0.95	0.74	1.87	7	pass
M. spicatum day 4 EC_{50}	3.70	−3.92	0.90	1.68	5.17	7	pass
M. spicatum day 7 EC_{50}	4.06	−3.92	0.96	1.60	4.47	6	pass
M. spicatum day 14 EC_{50}	4.91	−4.36	0.92	1.81	4.24	7	pass
M. spicatum day 28 EC_{50}	2.98	−2.32	0.96	0.55	2.23	7	pass
M. sibiricum day 4 EC_{10}	1.62	−1.37	0.85	0.09	1.13	7	pass
M. sibiricum day 7 EC_{10}	1.04	−0.38	0.70	0.002	0.14	7	fail
M. sibiricum day 7 EC_{10}*	3.16	−1.87	0.87	0.41	1.54	6	pass
M. sibiricum day 14 EC_{10}	2.24	−0.98	0.96	0.11	0.73	7	pass
M. sibiricum day 28 EC_{10}	4.28	−0.90	0.93	0.31	0.81	7	pass
M. sibiricum day 4 EC_{25}	2.38	−2.54	0.97	0.59	3.38	7	pass
M. sibiricum day 7 EC_{25}	5.98	−5.52	0.99	2.55	5.11	7	pass
M. sibiricum day 14 EC_{25}	2.94	−2.07	0.95	0.45	1.85	7	pass
M. sibiricum day 28 EC_{25}	3.96	−2.21	0.97	0.60	1.72	7	pass
M. sibiricum day 4 EC_{50}	2.88	−3.71	0.96	1.64	6.97	7	pass
M. sibiricum day 7 EC_{50}	6.67	−7.26	0.98	4.22	7.88	5	pass
M. sibiricum day 14 EC_{50}	3.11	−2.95	0.95	0.90	3.44	7	pass
M. sibiricum day 28 EC_{50}	3.24	−2.88	0.92	0.86	3.11	7	pass
Myriophyllum spp. day 4 EC_{10}	1.66	−1.00	0.95	0.06	0.68	14	pass
Myriophyllum spp. day 7 EC_{10}	3.04	−1.55	0.96	0.31	1.23	12	pass
Myriophyllum spp. day 14 EC_{10}	2.16	−0.58	0.98	0.07	0.47	14	pass
Myriophyllum spp. day 28 EC_{10}	4.09	−1.01	0.98	0.31	0.86	14	pass
Myriophyllum spp. day 4 EC_{25}	2.41	−2.13	0.96	0.40	2.25	14	pass
Myriophyllum spp. day 7 EC_{25}	4.21	−3.31	0.96	1.13	3.03	14	pass
Myriophyllum spp. day 14 EC_{25}	3.24	−1.94	0.94	0.44	1.60	14	pass
Myriophyllum spp. day 28 EC_{25}	4.79	−2.67	0.97	0.82	1.95	14	pass
Myriophyllum spp. day 4 EC_{50}	3.26	−3.83	0.95	1.69	6.05	14	pass
Myriophyllum spp. day 7 EC_{50}	4.99	−5.10	0.94	2.53	5.82	14	pass
Myriophyllum spp. day 14 EC_{50}	4.23	−3.89	0.97	1.55	4.14	12	pass
Myriophyllum spp. day 28 EC_{50}	3.45	−2.88	0.95	0.87	2.91	14	pass
L. gibba day 21 EC_{10}	2.39	−1.45	0.93	0.21	1.18	6	pass
L. gibba day 21 EC_{25}	3.47	−2.91	0.93	0.89	2.95	6	pass
L. gibba day 21 EC_{50}	4.52	−4.77	0.90	2.35	5.91	6	pass

[a] These values are transformed into units of log and probit for the purposes of regression and backtransforms were used to calculate the intercepts. The distribution units were in mg/L. [b] The toxicity threshold is the 0.1 centile of the toxicity distribution. [c] Number of data points used in the ranking.

available in spreadsheet form. The joint probability curve for an effect measure distribution from a single species can be interpreted as "under the described conditions, *x*% of effect measures (i.e., EC_{10}values) will show a response at *y*% of the current observations" (*6*).

Hazard Quotient Comparison. The toxicity thresholds calculated from the EC_{10} effect measure distributions were used in a HQ approach to assess the risk to these plants from MCA under field conditions. The HQ was calculated as

$$HQ = EEC/TBC \quad (3)$$

where TBC is the toxicological benchmark concentration (i.e., the toxicity threshold as calculated from the distributions) and the EEC is the highest expected environmental concentration. Values greater than 1 indicate a potential for toxic effects to occur, and values of less than 1 indicate that toxicity is not likely to occur (*3*) although there can be more rigorous interpretations depending on whether the test is chronic or acute (*50*). It was then compared to a HQ calculated for each plant using the most sensitive endpoint from MCA toxicity at the EC_{10} level (*27*), as a substitute for the NOEC (*14*, *15*), as the TBC.

Results

The effect measure distributions from single plant species, with the exception of *M. sibiricum* EC_{10} values after 7 days of exposure to MCA, were found to be log-normally distributed as defined by the *W*-test (Figure 1) (Tables 1 and 2). The exception was due to a single value, the node number EC_{10}, which when removed resulted in a log-normal distribution (Tables 1 and 2). The effect measures fit the log-normal distribution well, with r^2 values generally above 0.9.

When comparing the use of the Weibull (eq 1) or the Blom equations (eq 2), both appear to give equally well-fitted regressions. The Blom equation provided a less conservative estimate of toxicity, at both the 10th centile and the toxicity threshold level. Blom estimates that the toxicity thresholds and the 10th centile for the EC_{10} distributions are, on average, 1.8- and 1.2-fold greater, respectively. The 10th centile is provided as a means of characterizing the distribution as this centile is generally within the range of the plotted data and has been used as the risk assessment criterion in other PERAs (*4*).

Probabilistic risk assessment using distributions of exposures to MCA from Canadian and European waters (Table

TABLE 2. Regression Coefficients and Intercepts for Monochloroacetic Acid Field Toxicity Distributions for *Myriophyllum spicatum*, *M. sibiricum*, and *Lemna gibba* As Calculated Using the Blom Equation

distributions	$y = ax + b$[a]			regression intercepts (mg/L)[b]			
	a	*b*	r^2	toxicity threshold	10th centile	*n*[c]	*W*-test ($p > 0.05$)
M. spicatum day 4 EC_{10}	1.97	−0.69	0.94	0.06	0.50	7	pass
M. spicatum day 7 EC_{10}	2.74	−1.00	0.93	0.17	0.79	7	pass
M. spicatum day 14 EC_{10}	2.33	−0.23	0.96	0.06	0.20	7	pass
M. spicatum day 28 EC_{10}	3.71	−1.05	0.96	0.28	0.87	7	pass
M. spicatum day 4 EC_{25}	2.95	−2.08	0.96	0.45	1.86	7	pass
M. spicatum day 7 EC_{25}	3.98	−2.78	0.97	0.84	2.38	7	pass
M. spicatum day 14 EC_{25}	4.35	−1.95	0.94	0.55	1.42	7	pass
M. spicatum day 28 EC_{25}	5.23	−2.91	0.95	0.92	2.05	7	pass
M. spicatum day 4 EC_{50}	4.33	−4.59	0.91	2.22	5.81	7	pass
M. spicatum day 7 EC_{50}	4.83	−4.66	0.97	2.11	5.01	6	pass
M. spicatum day 14 EC_{50}	5.69	−5.06	0.91	2.22	4.61	7	pass
M. spicatum day 28 EC_{50}	3.50	−2.72	0.96	0.78	2.58	7	pass
M. sibiricum day 4 EC_{10}	1.89	−1.61	0.85	0.16	1.49	7	pass
M. sibiricum day 7 EC_{10}	1.23	−0.45	0.71	0.01	0.21	7	fail
M. sibiricum day 7 EC_{10}*	3.78	−2.24	0.88	0.60	1.79	6	pass
M. sibiricum day 14 EC_{10}	2.62	−1.15	0.97	0.18	0.89	7	pass
M. sibiricum day 28 EC_{10}	5.01	−1.06	0.95	0.39	0.90	7	pass
M. sibiricum day 4 EC_{25}	2.78	−2.96	0.87	0.90	4.02	7	pass
M. sibiricum day 7 EC_{25}	6.97	−6.09	0.99	2.69	4.90	7	pass
M. sibiricum day 14 EC_{25}	3.42	−2.41	0.95	0.63	2.14	7	pass
M. sibiricum day 28 EC_{25}	4.63	−2.59	0.98	0.78	1.92	7	pass
M. sibiricum day 4 EC_{50}	3.36	−4.32	0.96	2.32	8.02	7	pass
M. sibiricum day 7 EC_{50}	8.06	−8.78	0.98	5.08	8.52	5	pass
M. sibiricum day 14 EC_{50}	3.63	−3.44	0.95	1.25	3.93	7	pass
M. sibiricum day 28 EC_{50}	3.77	−3.35	0.91	1.17	3.54	7	pass
Myriophyllum spp. day 4 EC_{10}	1.83	−1.10	0.94	0.08	0.80	14	pass
Myriophyllum spp. day 7 EC_{10}	3.39	−1.72	0.96	0.39	1.35	12	pass
Myriophyllum spp. day 14 EC_{10}	2.39	−0.64	0.98	0.09	0.54	14	pass
Myriophyllum spp. day 28 EC_{10}	4.51	−1.12	0.98	0.37	0.92	14	pass
Myriophyllum spp. day 4 EC_{25}	2.65	−2.34	0.95	0.52	2.51	14	pass
Myriophyllum spp. day 7 EC_{25}	4.66	−3.66	0.96	1.33	3.24	14	pass
Myriophyllum spp. day 14 EC_{25}	3.56	−2.14	0.94	0.54	1.74	14	pass
Myriophyllum spp. day 28 EC_{25}	5.28	−2.94	0.97	0.94	2.06	14	pass
Myriophyllum spp. day 4 EC_{50}	3.59	−4.33	0.95	2.21	7.07	14	pass
Myriophyllum spp. day 7 EC_{50}	5.62	−5.74	0.94	2.96	6.21	14	pass
Myriophyllum spp. day 14 EC_{50}	4.67	−4.29	0.97	1.81	4.41	12	pass
Myriophyllum spp. day 28 EC_{50}	3.82	−3.18	0.96	1.06	3.14	14	pass
L. gibba day 21 EC_{10}	2.83	−1.71	0.93	0.33	1.42	6	pass
L. gibba day 21 EC_{25}	4.09	−3.43	0.92	1.21	3.35	6	pass
L. gibba day 21 EC_{50}	5.34	−5.65	0.90	3.02	6.58	6	pass

[a] These values are transformed into units of log and probit for the purposes of regression and backtransforms were used to calculate the intercepts. The distribution units were in mg/L. [b] The toxicity threshold is the 0.1 centile of the toxicity distribution. [c] Number of data points used in the ranking.

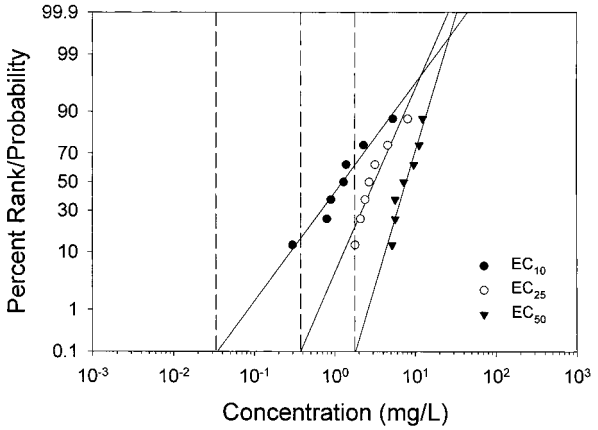

FIGURE 1. Monochloroacetic acid effect measure distributions generated from field level testing using the Weibull equation for *Myriophyllum spicatum* using day 14 EC_{10}, EC_{25}, and EC_{50} values. The dashed line represents the threshold of toxicity for that distribution.

3) with the effect measure distributions (Tables 1 and 2) showed a very low likelihood of exceedence of the effect measure distributions at the 0.1%, or threshold, level for any of the three plant species examined (Table 4) (Figure 2).

HQs based on a toxicity threshold for each plant species, calculated from intraspecies effect measure distributions at the 0.1% level, and the highest measured environmental concentration from data used in the exposure concentrations showed no hazard to these macrophytes resulting from current environmental exposures (Table 5). HQs for treated tap water were the highest, although always less than 1. These estimates were more conservative than those based on an EC_{10} from toxicity data and the highest environmental concentration. The use of the toxicity threshold calculated from the distribution of effect measures was 2.6−17.6-fold more protective than using the lowest EC_{10} alone. The exceptions to this were the *M. sibiricum* day 7 EC_{10} and combined *Myriophyllum* spp. day 7 EC_{10} distributions, where the lowest EC_{10} value, node number, was excluded from the ranking due to its skewing effect but was still used as the TBC. When these two distributions were excluded from the ratio analysis, the use of toxicity thresholds in the HQ assessment was, on average, 8-fold more protective than using the lowest EC_{10} as the TBC.

TABLE 3. Regression Coefficients and Intercepts for Monochloroacetic Acid Environmental Concentrations in Canadian Lake, River, and Drinking Waters and Swiss River Waters As Calculated Using the Weibull Equation

group	$y = ax + b$[a]			regression intercepts (ng/L)	
	a	*b*	r^2	90th centile	n[b]
Canadian lakes	2.24	9.87	0.97	147	29
Rhein River, Switzerland	3.83	15.61	0.95	182	32
Rhone River, Switzerland	1.85	7.76	0.93	309	17
Swiss rivers	2.58	10.55	0.93	256	79
Brittannia, ON, Canada river water	3.00	10.11	0.86	1140	11
Brittannia, ON, Canada treated water	3.38	9.26	0.90	4360	13
Hull, PQ, Canada river water	4.28	14.17	0.95	974	13
Hull, PQ, Canada treated water	3.06	8.48	0.75	4441	13
Buckingham, PQ, Canada river water	2.76	9.68	0.97	905	12
Buckingham, PQ, Canada treated water	2.81	8.35	0.97	3052	8

[a] These values are transformed into units of log and probit for the purposes of regression and backtransforms were used to calculate the intercepts. The distribution units were in mg/L. [b] Number of data points used in the ranking.

TABLE 4. Probability of Exceeding Toxicity Threshold for Monochloroacetic Acid Calculated from Macrophyte Effect Measure Distributions at EC_{10}, Both the Weibull and Blom Equations, for Various Exposure Distributions

exposure distribution	macrophyte effect measure distributions (%)[a]			
	Lemna gibba	*Myriophyllum spicatum*	*Myriophyllum sibiricum*	*Myriophyllum* spp.
Canadian lakes	≪0.01	≪0.01	≪0.01	≪0.01
Rhein River	≪0.01	≪0.01	≪0.01	≪0.01
Rhone River	≪0.01	≪0.01	≪0.01	≪0.01
Swiss rivers	≪0.01	≪0.01	≪0.01	≪0.01
Canadian water treatment plants (raw)	≪0.01	≪0.01	≪0.01	≪0.01
Canadian water treatment plants (treated)	≪0.01	≪0.01	≪0.01	≪0.01

[a] Includes all the dates evaluated.

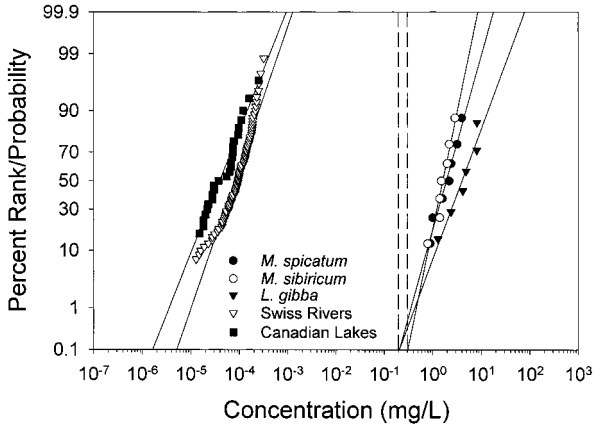

FIGURE 2. Exposure distributions of monochloroacetic acid (MCA) concentrations in Canadian lakes, modified from ref *31*, and Swiss rivers, modified from ref *30*, and MCA effect measure distributions generated from field-level testing using the Weibull equation of three macrophyte species; *Myriophyllum spicatum* and *M. sibiricum* using 28 day EC_{10} values and *Lemna gibba* using 21 day EC_{50} values. The dashed line represents the threshold of toxicity for that distribution.

Discussion

The concentration−response data generated from the field study with MCA (*27*) allowed for the plotting of cumulative frequency distributions of effect measures for individual species of aquatic plants. The distributions were found to be log-normal and are assumed to contain all effect measures not examined during the course of the study. The distributions were used to estimate thresholds of toxicity, which was defined as a low centile (0.1%) of the distribution. These thresholds were interpreted as the concentrations at which no impacts should be observed for any endpoint above the specific effect level plotted. An example would be if the distribution plotted was constructed from EC_{25} values, then the threshold is the concentration beyond which no impacts greater than 25% change from control should be observed for any endpoint. Since acute data tends to be limited to mortality, these distributions are likely best constructed from chronic toxicity data. Care should be taken in the final interpretation of the estimated toxicity thresholds, as they are based on values extrapolated beyond the actual plotted data.

By plotting the field-derived EC_{10} values of various endpoints for a toxicant in this fashion and using the distribution in a modified probabilistic ecological risk assessment or the estimated threshold of toxicity in the calculation of a HQ, the risk assessor can be more confident that responses are unlikely to occur in these nontarget aquatic plants. The use of field-derived data in risk assessment is advantageous as it provides a more realistic estimate of toxicity as normal degradation and partitioning of toxicants can occur as compared to laboratory data, which can result in an overestimation of adverse impacts (*6*). The drawback of using lab-based data from the phytotoxicity assay for *Myriophyllum* spp. in risk assessments has been noted by Roshon et al. (*20*).

Using both HQ and PERA techniques with current environmental concentrations of MCA in Canadian and European surface waters (*30*, *31*, *45*) and the distributions of effects plotted for these three plant species and the genus *Myriophyllum* spp., there is an essentially negligible likelihood of any effects occurring. Concentrations of MCA in tap water may pose a risk to these plants based on the HQ assessment, but exposure through this route is unlikely.

Using the standard HQ approach, the calculation of a threshold of toxicity from the distributions produced a more conservative estimate of possible response as compared with the use of the lowest EC_{10} value. The average ratio of the two

TABLE 5. Hazard Quotients for Aquatic Macrophytes *Myriophyllum spicatum*, *M. sibiricum*, and *Lemna gibba* As Calculated from Toxicity Threshold of the EC_{10} Effects Distributions and Exposure from Canadian (250 ng/L), Swiss Surface Waters (320 ng/L), Canadian Treatment Intake Water (1200 ng/L) and Treated Water (7800 ng/L) As Compared to Those Calculated Using the Lowest Calculated EC_{10} for Each Plant and Time Point

			hazard quotients using toxicity threshold				
effect distribution	toxicity threshold (μg/L)	lowest EC_{10} (μg/L)	Canadian lakes	Swiss rivers	tap water intake	treated tap water	ratio[a]
M. spicatum day 4	30	600	0.007	0.009	0.035	0.229	17.6
M. spicatum day 7	110	1000	0.002	0.003	0.011	0.071	9.1
M. spicatum day 14	20	300	0.013	0.016	0.060	0.390	15.0
M. spicatum day 28	200	900	0.001	0.002	0.006	0.039	4.5
M. sibiricum day 4	90	900	0.003	0.004	0.014	0.087	10.0
M. sibiricum day 7	410	100	0.001	0.001	0.003	0.019	0.2
M. sibiricum day 14	110	700	0.002	0.003	0.011	0.071	6.4
M. sibiricum day 28	310	800	0.001	0.001	0.004	0.025	2.6
Myriophyllum spp. day 4	60	600	0.004	0.005	0.020	0.130	10.0
Myriophyllum spp. day 7	310	100	0.001	0.001	0.004	0.025	0.3
Myriophyllum spp. day 14	70	300	0.004	0.005	0.017	0.111	4.3
Myriophyllum spp. day 28	310	800	0.001	0.001	0.004	0.025	2.6
L. gibba day 21	210	1300	0.001	0.002	0.006	0.037	6.2

[a] The ratio of the HQ with the threshold of toxicity as the TBC and HQ (not shown) with the lowest EC_{10} as the TBC.

calculated HQs tested was 8, which is surprisingly close to the commonly applied uncertainty factor of 10 used in most regulatory environmental risk assessments (*51*). This implies that the thresholds of toxicity from these distributions, in general, provide a reasonable level of protection when compared with current methodologies to estimate uncertainty.

The distributions can also be used to compare the sensitivities of the different plants species. Using the day 14 EC_x distributions at the 10th centile as a means of comparison, *M. spicatum* appears to be marginally more sensitive than *M. sibiricum*. *M. spicatum* is more sensitive at the 10th centiles for the EC_{10} and EC_{25} distributions, but *M. sibiricum* is slightly more sensitive at the EC_{50} distribution. The slopes themselves are also indicative of the variation in sensitivity of the different species. The smaller the slope, the greater range of sensitivity for the various effect measures that the plant exhibits. They could also be used to compare between different exposure durations, such as 4 day vs 28 day, between different toxicants, and between field and laboratory tests.

The use of single species sensitivity distributions of effect measures has a number of possible further applications. If enough species are tested for a single compound and distributions can be plotted, the individual thresholds of toxicity could be combined and plotted from each individual species in much the same manner as a conventional species sensitivity distribution. In this manner, each point in the species sensitivity distribution would represent a toxicity threshold for a single species. The distribution could be used to predict what proportion of species would have their toxicity thresholds exceeded. Since the toxicity thresholds are based on extrapolated values, the use of a higher centile, such as the 1st or 10th, may be more appropriate as these tend to be within the observed values, and there will be greater confidence in their representativeness.

This method also has the potential to evaluate mixture toxicity within a single species. This may be done by determining the distribution of effect measures for two compounds independently and then examining the toxicity of the mixture of the two combined. If two distributions for the EC_{50} values or other effect level are normalized as toxic equivalents based on the more toxic of the two compounds, then they can be plotted on the same graph. The individual toxicity values for specific effect measures would be combined by dividing each by one-half and adding the two results together. These would then be plotted in the same manner as previously described to produce a predicted distribution

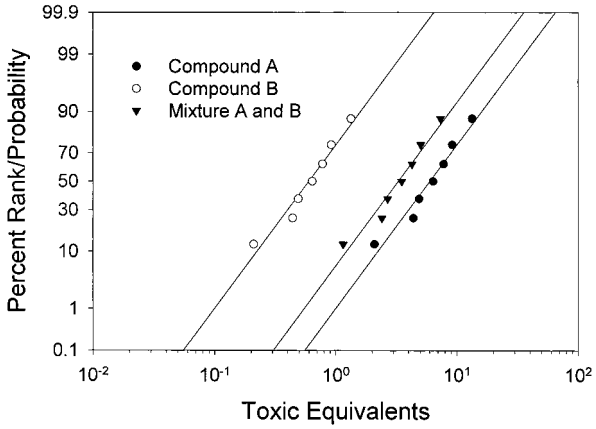

FIGURE 3. Idealized distributions for two single compounds with the *x*-axis normalized to the more toxic of the two and their combined distributions representing the predicted distribution for an equitoxic mixture of the two compounds. Actual distributions to the right of the predicted would indicate antagonism while distributions to the left would indicate synergism.

for mixture toxicity (Figure 3). This would represent a distribution of an additive interaction between the two compounds. The mixture toxicity test would use toxic equivalents to define the concentration−response curve and calculate the EC_x values for plotting of the observed, or actual, mixture distribution. The regressions from the two distributions can be tested for significant differences of the slope and intercept (*52*). Significant deviation from the predicted model distribution by the actual distribution would indicate interactions other than additivity. Distributions shifted to the right of the predicted model would be exhibiting antagonistic interactions, and those to the left would be exhibiting synergistic interactions. An assumption is that the effect measures for each compound show the same order of sensitivity and that there are the same number of calculated effect measures in the distributions. In this way, a regression analysis can be performed on the predicted mixture distribution for comparison with the actual distribution. These assumptions still need to be tested and verified.

The use of plants in this type of analysis is fairly simple when compared to other organisms. Plants, both aquatic and terrestrial, have numerous effect measures that can be evaluated simultaneously and efficiently. Other possible effect measures that could be investigated but were not included

in these analyses include flowering, seed production, seed viability, root mass (both fresh and dry), leaf area, root area, and pollen production (*19*, *20*). This suite of effect measures may not be readily gathered in other organisms, but enough could be gathered to provide the basis for a distribution. In the FETAX assay with the amphibian *Xenopus laevis* (*53*), measurements on egg survival, time to hatch, growth rates such as mass and length, deformities, and mobility might be enough to provide a distribution of responses. When considering the use of an effect measure, it should exhibit a concentration or dose−response. While it is possible to analyze many biochemical and enzymatic systems for perturbations, these may not respond in a concentration−response fashion or may be confounded by other factors so that the true impact is masked (*27*, *54*), so care should be taken in their inclusion in a distribution.

In conclusion, MCA at current environmental concentrations does not appear to be a risk to aquatic macrophytes. It is broken down in aquatic environments (*27*, *55*) and is therefore unlikely to increase in concentration over time as has been observed with other HAAs (*23*, *55*−*57*). The use of *Myriophyllum* spp. in ecotoxicological risk assessment, in the context of this study, is promising. These plants can be evaluated for a wide variety of effect measures and develop well under semi-natural field conditions (*27*, *57*). The use of single species effect measure distributions for aquatic plants appears to be a useful approach for assessing the risk to these organisms from contaminants. It could be a powerful new tool when examining the potential for toxicity to a rare or ecologically important plant, such as a keystone species. In the future, this method should be applied to data generated from species in other classes of organisms, such as fish, invertebrates, amphibians, and different plant species, to test its general utility to estimate thresholds of toxicity.

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