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Macrophytes are highly sensitive to the herbicide diquat dibromide in test systems of varying complexity

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ABSTRACT

The herbicide diquat dibromide is used in North America to manage nuisance macrophytes. However, its effect on native macrophytes is less clear and it could cause indirect effects on other aquatic biota. This study determined the sensitivity of both native and non-native macrophytes grown in test systems with varying complexity to diquat dibromide applied directly to water following label directions. In an outdoor mesocosm experiment and single species greenhouse concentration-response tests, *Elodea canadensis* Michx., *Myriophyllum spicatum* L., *Ceratophyllum demersum* L. and *Hydrocharis morsus-ranae* L. were exposed to a range of diquat dibromide concentrations (4.7 – 1153 µg/L), corresponding to 0.4 – 100% of the recommended label rate of the formulated product. The mesocosm experiment contained all four plant taxa in the same system along with caged amphipods (*Hyalella azteca* Saus.), tadpoles (*Lithobates pipiens* Schreber), phytoplankton and periphyton; however, this study focuses on the macrophytes only. In both test systems, severe direct effects of diquat dibromide on macrophytes were detected, with almost 100% mortality of all macrophytes in both test systems at 74 µg/L. The most sensitive species in the single species tests, *E. canadensis*, showed almost 100% mortality at concentrations below the HPLC-based method detection limit of 5 µg/L. Effects occurred very rapidly and showed no difference in severity between native and non-native macrophytes or complexity of test systems. These results suggest that diquat dibromide could be applied at a considerably lower label rate, depending on the characteristics of the waterbody, while still achieving effective control of nuisance macrophytes.

1. Introduction

Aquatic herbicides such as diquat dibromide are applied directly to waterbodies to manage nuisance macrophytes in North America. Diquat dibromide is inactivated quickly in the presence of organic matter and vegetation; hence, it has been approved for use in aquatic habitats as it is thought to pose minimal long-term risks to non-target aquatic biota (Davies and Seaman, 1968; Emmett, 2002; US EPA, 1995; Wilson and Wu, 2012). However, diquat dibromide is both adsorbed onto and absorbed into vegetation (Davies and Seaman, 1968), which results in long-term accumulation and possible release when contaminated plants die and decompose. Moreover, due to its high efficacy (Johnson, 1965), the native non-target plant community is likely to also be affected. Such removal of vegetation could dramatically change the ecosystem, resulting in indirect effects on other trophic levels including invertebrates, amphibians and fish (Berry, 1984; May et al., 1973; Nicholson and Clerman, 1974).

The current regulatory risk assessment for herbicides in Canada, the United States and the European Union relies mainly on toxicity tests with algae (*Raphidocelis subcapitata*) and duckweed (*Lemna* spp.) because these taxa are easy to culture and show uniform and rapid growth (Lewis, 1995; Maltby et al., 2010). However, scientific and regulatory communities have raised concerns that a risk assessment based on these toxicity tests may not be protective for other macrophyte species with differing physiology and morphology (Davy et al., 2001; Maltby et al., 2010; Mohr et al., 2013). The sensitivity of plants to herbicides is species-specific and no macrophyte is consistently the most sensitive in studies comparing toxicity (Arts et al., 2008; Fairchild, 1998; Giddings

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et al., 2012; Lewis, 1995; Vervliet-Scheebaum et al., 2006). The commonly used *Lemna minor* L. is typically of intermediate sensitivity (Cedergreen et al., 2004; Fairchild et al., 1998). Depending on the herbicide tested, *Lemna* spp. can be less sensitive compared to *Ceratophyllum demersum* L., *Elodea canadensis* Michx. and *Myriophyllum* spp. (Cedergreen et al., 2004; Fairchild et al., 1998; Perkins, 1997; Teodorović et al., 2012).

Diquat dibromide could affect a range of macrophytes growing in the application area when dissipating from the water surface to the sediment, because it is a non-selective, highly water-soluble contact herbicide that inhibits photosynthesis (Dodge and Harris, 1970; Funderburk and Lawrence, 1964). Given this non-selective mode of action and potential exposure of sensitive submerged and rooted macrophytes, toxicity tests with algae and duckweed may be insufficient to evaluate toxic effects on other non-target macrophytes. Ecotoxicity tests with a suite of floating, submerged and rooted macrophytes can increase risk assessment accuracy (Arts et al., 2008; Davy et al., 2001; Fairchild et al., 1997; Giddings et al., 2012; Lewis, 1995; Maltby et al., 2010; Vervliet-Scheebaum et al., 2006). The submerged macrophyte taxa *Myriophyllum* spp., *Elodea* spp. and *Ceratophyllum* spp. have been identified and recommended as additional test species (Davy et al., 2001; European Commission, 2013; Maltby et al., 2010), however, standard single species test guidelines are currently only available for *Myriophyllum spicatum* L. (OECD, 2014a, 2014b).

This study determined the sensitivity of macrophytes to diquat dibromide, directly applied to water following label recommendations (Syngenta Canada Inc, 2015) in test systems of varying complexity to provide phytotoxicity data for risk assessment. The test species chosen include *E. canadensis*, *M spicatum*, *C. demersum* and *Hydrocharis morsus-ranae* L. and thus represent a range of floating, submerged and rooted native and non-native macrophytes that are common in North American waterbodies. We assessed the phytotoxicity of diquat dibromide in an outdoor mesocosm experiment and single species greenhouse concentration-response tests to cover two tiers of herbicide risk assessment (Boutin et al., 1995; Davy et al., 2001; EFSA PPR Panel, 2013; Solomon et al., 2008; US EPA, 2016). The single species tests evaluated effects of diquat dibromide on macrophytes grown in small systems without interactions with other species. The mesocosm experiment assessed the effects of diquat dibromide to macrophyte assemblages on a larger scale, and also determined risks to aquatic biota including phytoplankton and periphyton, caged amphipods and tadpoles. However, the current study focuses solely on the effects of diquat dibromide on macrophytes in these mesocosms. Using two test systems based on different spatial scales and ecological complexity provides a more realistic estimate of a given pesticide's fate and effects and thus a sounder basis for risk assessment decisions (Maltby et al., 2010; Sanderson, 2002; van den Brink, 2013).

2. Materials and methods

2.1. Test species

The four macrophyte test species *E. canadensis* (native to North America, submerged, rooted), *M. spicatum* (non-native, submerged, rooted), *C. demersum* (native, submerged, non-rooted) and *H. morsus-ranae* (non-native, floating) were obtained from natural populations in lakes and rivers in Ontario, Canada (Table A.1) with no known prior or current diquat dibromide applications. *E. canadensis*, *M. spicatum* and *C. demersum* plant material was collected in summer 2016; *H. morsus-ranae* plants were grown from turions that had been collected in fall 2015 and stored in water at 4 °C in the dark. All plants were rinsed in fresh water to eliminate visible contamination with algae and invertebrates and kept in an outdoor stock tank for acclimatization for two weeks.

2.2. Test substance

Diquat dibromide (6,7-dihydrodipyrido[1,2-a:2′1'-c]pyrazinediium dibromide) was applied as the formulated product Reward® Aquatic Herbicide (Syngenta Canada Inc, 2015). Applying the formulation is more environmentally relevant than applying the technical grade product because it represents the actual product applied to natural ecosystems and includes dispersants or other additives that may affect toxicity (Mesnage et al., 2014). Reward® Aquatic Herbicide can be used in a variety of waterbodies with still or slow flowing water, such as dugouts, ponds, ditches, lakes, streams and canals (Syngenta Canada Inc, 2015). A specific label application rate is recommended for control of regular macrophyte growth in all waterbodies of ≤ 1.5 m depth (18.3 L/ha), which we considered for our mesocosms (120 cm length × 78 cm width × ~55 cm depth). This label rate for control of regular macrophyte growth (18.3 L/ha), and the label rate for early stages of macrophyte growth (9.2 L/ha) (Syngenta Canada Inc, 2015) were converted into µg/L active ingredient diquat dibromide, taking into account the surface area (0.735 m^2) and volume (280 L) of the mesocosms, as aquatic ecotoxicology studies commonly report toxicities in mass per volume units such as µg/L. This resulted in diquat dibromide label rate concentrations of 1153 µg/L (18.3 L/ha) and 579 µg/L (9.2 L/ha) active ingredient, respectively.

The test concentrations were determined following a geometric series. The two highest concentrations were selected from the label rates for regular macrophyte growth (1153 µg/L) and for early stages of macrophyte growth (579 µg/L). The subsequent lower concentrations followed a geometrical progression with the factor of ~2 calculated by dividing 1153 µg/L by 579 µg/L. In the mesocosm experiment, diquat dibromide was tested at five nominal concentrations (1153, 579, 291, 147 and 74 µg/L) plus controls, with five replicates each, for a total of 30 mesocosms. It was not logistically possible to incorporate additional lower concentrations, as each mesocosm required manipulations and monitoring daily. Nominal test concentrations of the single species concentration-response tests were 1153, 579, 291, 147 and 74 µg/L, and the additional lower concentrations 37, 19, 9 and 4.7 µg/L. These test concentrations correspond to a range of 100 – 0.4% of the label application rate for regular macrophyte growth, as described above.

Single species tests with treatments corresponding to the mesocosm treatments (1153, 579, 291, 147 and 74 µg/L) were initiated shortly after the mesocosm experiment, but were only performed for *E. canadensis* and *H. morsus-ranae*, because early results from the mesocosm experiment indicated that an almost 100% effect at all treatment concentrations was likely. Consequently, tests for *M. spicatum* and *C. demersum*, and a second test for *E. canadensis* were performed at a range of lower concentrations (74, 37, 19, 9 and 4.7 µg/L), still providing overlap with the higher concentration range for confirmation purposes. Additional tests with *H. morsus-ranae* were not conducted due to lack of available plant material.

For verification of the nominal concentrations, water samples (1 L) were collected from the mesocoms 1 h post application, and samples were taken from single species test stock solutions. Samples were analyzed by a commercial, accredited (Canadian Association for Laboratory Accreditation Inc.) laboratory, Caduceon Environmental Laboratories (285 Dalton Avenue, Kingston, Ontario K7K 6Z1, Canada), using high performance liquid chromatography as described in the US EPA Method 549.1 (US EPA, 1992). Nominal concentrations were verified for both the mesocosm experiment (76% – 88% of respective nominal concentrations, Table A.2) and the single species tests (90% – 151% of respective nominal concentrations, Table A.2). The discrepancies in percent nominal recovery between mesocosm and single species tests are likely due to differences in: 1. sampling techniques, as some adsorption would be expected in the mesocosms 1 h after application, which would not occur when sampling the stock solutions from single species tests, and 2. concentration range, as the lower concentration range in single species tests means that smaller absolute