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Evaluating Sensitivity of Five Aquatic Plants to a Novel Arylpicolinate Herbicide Utilizing an Organization for Economic Cooperation and Development Protocol

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New arylpicolinate herbicide chemistry under development for rice, aquatic weed management, and other uses was evaluated using five aquatic plants. The herbicide 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester—also identified as XDE-848 BE or SX-1552 (proposed International Organization for Standardization common name in review; active tradename Rinskor^{TM})—and its acid form (XDE-848 acid or SX-1552A) were evaluated on three dicots: (1) Eurasian watermilfoil (EWM), (2) megalodonta, and (3) crested floating heart (CFH), and two monocots: (1) hydrilla and (2) elodea. A small-scale Organization for Economic Cooperation and Development (OECD) protocol developed using EWM for registration studies was utilized. EWM and megalodonta were also evaluated in larger-scale mesocosms for comparison. In-water concentrations between 0.01 and 243 μg ai L^{-1} as SX-1552 or SX-1552A were applied under static conditions for 14 (growth chamber) or 28 d (mesocosm). EWM was susceptible to both SX-1552 and SX-1552A, with dry-weight 50% effective concentration (EC_{50}) values of 0.11 and 0.23 μg ai L^{-1} under growth chamber conditions. Megalodonta had EC_{50} values of 11.3 and 14.5 μg ai L^{-1} for the SX-1552 and SX-1552A. CFH was more sensitive to SX-1552 (EC_{50} = 5.6 μg ai L^{-1}) than to SX-1552A (EC_{50} = 23.9 μg ai L^{-1}). Hydrilla had EC_{50} values of 1.4 and 2.5 μg ai L^{-1}, whereas elodea was more tolerant, with EC_{50} values of 6.9 and 13.1 μg ai L^{-1} for SX-1552 and SX-1552A, respectively. For EWM mesocosm trials, EC_{50} values for SX-1552 and 1552A were 0.12 μg ai L^{-1} and 0.58 μg ai L^{-1}, whereas the megalodonta EC_{50} was 6.1 μg ai L^{-1}. Activity of SX-1552 on EWM, hydrilla, and CFH merits continued investigation for selective aquatic weed control properties. Results suggest that the OECD protocol can be used to screen activity of herbicides for multiple aquatic plant species.

Nomenclature: 4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester; crested floating heart, *Nymphoides cristata* (Roxb.) Kuntze; elodea, *Elodea canadensis* Michx.; Eurasian watermilfoil, *Myriophyllum spicatum* L.; hydrilla, Hydrilla verticillata L.f. *Royle*; megalodonta, *Bidens beckii* Torr. Ex Spreng.

Key words: Aquatic herbicide, aquatic plant bioassay, aquatic plant toxicity, Beck's water-marigold, herbicide screening, invasive aquatic plants.

Aquatic weed control with herbicides is characterized by unique conditions and management objectives vs. agricultural or other terrestrial weed management (APMS 2014). Perhaps the two most significant differences in use of aquatic vs. terrestrial herbicides are (1) labeled use for direct application into water to achieve a target herbicide concentration and exposure and (2) high standards for targeting an invasive or nuisance plant with limited impact to multiple native or desirable plant species. In the typical agricultural setting direct application to water is prohibited and broad-spectrum weed control is provided for a single nontarget species. Aquatic herbicide registration by the U.S. Environmental Protection Agency and other international regulatory agencies requires demonstration of negligible risks to human health or the environment.

Risk assessments of aquatic herbicides consider human water uses and exposure (e.g., drinking, recreational use including swimming, and irrigation practices), other incidental exposure routes, and possible impact to nontarget biota: algae, fish, invertebrates, and nontarget aquatic vegetation. Stringent requirements for aquatic herbicide registration have limited the number of active ingredients approved for aquatic use. Although 244 herbicide active ingredients are currently registered in the United States, only 14 are registered as aquatic herbicides (NPIRS 2015). There is a technical need for additional

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herbicides and alternative modes of action for aquatic weed management. New herbicides can improve response to new aquatic invaders, enhance selectivity to desirable native aquatic vegetation, reduce use rates, and mitigate risk of potential herbicide resistance development (APMS 2014; Getsinger et al. 2008).

To support the development of a potential new aquatic herbicide, a new chemistry was screened against several target and nontarget aquatic plants. The herbicide 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-pyridine-2-benzyl ester, is under development by Dow AgroSciences for rice (XDE-848 BE;proposed International Standardization Organization common name in review; active tradename Rinskor™) and other agricultural crops and is also under development in partnership with SePRO Corporation as an aquatic herbicide (SX1552; Procellacor™; Aquatic Herbicide Technology System). SX-1552 is a member of a new class of synthetic auxins in the arylpicolinate herbicide family. In preliminary screening, SX-1552 exhibited efficacy on several invasive U.S. aquatic weeds including the submersed plants hydrilla and EWM, and the floating-leaf plant CFH (SePRO Corporation, unpublished data). SX-1552 would represent a new chemical class for aquatic uses. Studies of *Arabidopsis thaliana* with mutations in select auxin-binding receptor proteins, along with direct molecule–protein interaction testing of these same receptor proteins, support that arylpicolinate chemistry including SX-1552 has a different binding affinity vs. 2,4-D and other synthetic auxins currently registered as herbicides (Bell et al. 2015; Lee et al. 2013; Villalobos et al. 2012; Walsh et al. 2006).

Laboratory studies and preliminary field dissipation studies indicate that SX-1552 in water is subject to rapid photolysis—a common mechanism of breakdown for several aquatic herbicides. SX-1552 can also convert partially via hydrolysis to an acid form (SX-1552A) with suspected reduced herbicidal activity.

Small-scale evaluation methods serve multiple purposes in aquatic herbicide development including characterization of relative activity for a particular mode of action and determination of weed spectrum including information on efficacy and selectivity. Several different small-scale methods have been utilized to characterize herbicidal activity on aquatic plants. Historically, baseline toxicity tests on duckweed (*Lemna* spp.) have driven regulatory assessment of pesticide risks to nontarget vascular aquatic plants (OECD 2006, USEPA 2012). Past small-scale laboratory testing to predict aquatic herbicide activity has included analysis of photosynthetic pigment concentrations after exposure to carotenoid biosynthesis inhibitors such as fluridone and topramezone (Berger et al. 2015; Glomski and Netherland 2011; Netherland et al. 1993). Contact aquatic herbicide activity for endothall (protein phosphate inhibitor), diquat (photosystem I inhibitor), flumioxazin, and carfentrazone (Protox inhibitors) have been quantified using conductivity testing of ion leakage (Glomski and Netherland 2013; Koschnick et al. 2006; MacDonald et al. 1993). For the auxin herbicides 2,4-D and triclopyr, controlled laboratory and greenhouse studies have defined concentration–exposure time relationships for EWM control (Green and Westerdahl 1990, Netherland and Getsinger 1992) and nontarget aquatic plant activity (Belgers et al. 2007; Hofstra and Clayton 2001; Netherland and Glomski 2014; Sprecher et al. 1998; Sprecher and Stewart 1995) that have been predictive of selective EWM control observed in the field (Nault et al. 2014; Parsons et al. 2001, Poovey et al. 2004, Wersal et al. 2010).

On the basis of the successful correlation of laboratory and mesocosm-scale studies and field evaluations with currently registered auxin-mimic aquatic herbicides, aquatic use pattern development for SX-1552 can be accelerated through initial data generation of laboratory-scale efficacy and selectivity. Realism of small-scale testing methodology for determinations of herbicidal efficacy, selectivity, and general ecological risk assessment is debated (Maltby et al. 2010). In 2014, a small-scale testing protocol using EWM was adopted by the OECD as a method to generate additional data for assessment of potential nontarget aquatic plant effects when *Lemna* spp. are not sensitive to the mode of action (OECD 2014). OECD method test results on EWM are now used in risk assessments supporting the registration of certain herbicidal modes of action in the European Union. There is minimal published data for aquatic herbicides that directly compare results of "microscale" laboratory screening with outcomes of larger-scale controlled studies using more established plants—typically at an aquarium or mesocosm scale under greenhouse or outdoor conditions. The OECD protocol (2014) describes the guidelines surrounding water and sediment testing for impacts of pesticides on rooted EWM. The results are used for registration purposes in Europe, and EWM was selected as the preferred species in cases where data are required for specific herbicidal modes of action or for a submerged, rooted dicotyledonous plant. The guidelines provide specifications

for creating a sediment and water source used in the studies (OECD 2014; Smart and Barko 1985). Although the focus of the OECD protocol is on EWM sensitivity and risk assessment for registration, the potential for using this small-scale assay to test other submersed plant species or to test new herbicides for aquatic plant activity has not been evaluated. Potential benefits of using the OECD protocol as an initial screen for testing aquatic herbicides against multiple species of plants include: (1) small space requirements allow for significant replication; (2) use of rooted plants allows for increased confidence in efficacy testing; (3) protocol can be easily modified to fit research objectives; and (4) use of standard water and sediments will allow for improved comparison of results across laboratories.

The first objective of this study was to evaluate SX-1552 and SX-1552A against five submersed plant species (three dicots and two monocots) to confirm and compare activity and potential utility as an aquatic herbicide. The second objective was to determine if the growth chamber studies provided comparable results with larger-scale mesocosm trials. The third objective was to determine the potential utility of the OECD protocol for screening different herbicides or additional plant species.

Materials and Methods

EWM from the Crystal River, FL, dioecious hydrilla from Lake Cypress, FL, CFH from Lake Okeechobee, FL, and megalodonta (water marigold) and elodea from Lake Minnetonka, MN were utilized for growth chamber and greenhouse trials. Plants were grown in culture tanks at the University of Florida Center for Aquatic and Invasive Plants (Gainesville, FL) for use in studies. Stock cultures were maintained under ambient outdoor conditions, and robust growth was noted for all species through the evaluation period from September through April.

Growth Chamber Trials. In this study, the OECD protocol was utilized for evaluating the response of the dicots, EWM, megalodonta, and CFH, and the monocots, elodea and hydrilla, after SX-1552 applications to the water under controlled conditions.

Apical shoot tips of 6 cm in length were collected from culture tanks and thoroughly rinsed to remove epiphytes or carbonate crusts on the leaf tissue. Four apical shoots of a single species were each planted into 250-ml beakers containing 200 ml of sediment specified in the protocol (OECD 2014). At least 3 cm of the shoot were pushed into the sediment. The 250-ml beakers containing sediment and plants were then placed in 2-L beakers containing 1.75 L of culture water (Smart and Barko 1985). The 2-L beakers were then placed in Percival E-36L environmental growth chambers set to a temperature of 21 C, a photoperiod of 16 light (L) : 8 dark (D), and light intensity of 275 $\pm$ 27 μmol m^{-2} s^{-1}. For the hydrilla and CFH trials, the temperature was increased to 25 C to facilitate improved plant growth.

All plants were given a pretreatment establishment period ranging from 9 to 11 d. This allowed for an increase in shoot tissue and root formation at the nodes of tissue buried in the sediment before treatment. To determine if root formation was present, selected beakers were removed and checked for roots. Before initiating treatments, multiple root formation was observed for all species. The pretreatment pH of the water was within OECD specifications (7.5 to 8.0). Pretreatment measurements on shoot fresh weight, dry weight, and total stem length (including lateral shoots) were collected by removing one plant from each of the beakers (three apical shoots remained). As the expected response to SX-1552 was unknown for these species, nonreplicated range-finding studies were conducted to determine concentrations that would be evaluated for each species (data not shown).

Both the SX-1552 (herbicide formulation analytically validated 300 g ai L^{-1} suspension concentrate) and SX-1552A (analytical grade) were provided by the SePRO Corporation (Carmel, IN) and evaluated against EWM, megalodonta, CFH, elodea, and hydrilla. Stock solutions of both SX-1552 and SX-1552A were created for treatment of the 2-L beakers. Herbicide concentrations for growth chamber experiments are listed in Table 1. Once treated, static conditions were maintained over the 14-d incubation period. Deionized water was added to the beakers to replace water lost to evaporation. Entire plants were harvested at 14 d after treatment (DAT) and dried to a constant weight at 70 C for a minimum of 48 h.

Prior herbicide concentration monitoring and the lack of UV light in the growth chambers indicated limited potential for photolytic breakdown of SX-1552 in this test system. Water samples ($\sim$ 25 ml) were collected immediately after treatment and 1, 7, and 14 DAT in selected treatment beakers to determine initial and final exposure concentrations. Samples were analyzed via high-performance liquid chromatography with tandem mass spectroscopy with limits of quantitation of 0.02 μg ai L^{-1} for SX-1552 and 0.05 μg ai L^{-1} for SX-1552A. Each

Table 1. Overview of SX-1552 and SX-1552A concentrations used in growth chamber and mesocosm studies.

Plant species tested	Concentrations evaluated	Material tested
	μg L^{-1}	
Growth chamber studies		
Eurasian watermilfoil (dicot)	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 9, 27, and 81	SX-1552 and SX-1552A
Water marigold (dicot)	0, 0.3, 1, 3, 9, 27, 81, and 243	SX-1552 and SX-1552A
Crested floating heart (dicot)	0, 1, 3, 9, 27, and 81	SX-1552 and SX-1552A
Hydrilla (monocot)	0, 0.3, 1, 9, 27, and 81	SX-1552 and SX-1552A
Elodea (monocot)	0, 0.1, 0.3, 1, 3, 9, 27, and 81	SX-1552 and SX-1552A
Greenhouse studies		
Eurasian watermilfoil	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 9, and 27	SX-1552 and SX-1552A
Water marigold	0, 0.1, 0.3, 1, 3, 9, 27, and 81	SX-1552

treatment was replicated four times and each study was repeated.

Mesocosm Trials. Both EWM and megalodonta were evaluated under greenhouse conditions from October to December, 2015 to determine impact of SX-1552 on more established plants. For EWM, two studies using both the herbicide formulations of SX-1552 and SX-1552A were conducted, whereas only SX-1552 was tested for megalodonta. A series of 3.78-L pots was filled with Margo Professional topsoil (92% sand, 4% silt, 4% clay) amended with 1 g of fertilizer (Osmocote® 15–9–12) kg^{-1} of soil. Four apical shoots (10 cm) of each test species were planted in individual pots and placed in 95-L plastic tanks filled with well water. The plants were given a 28-d pretreatment establishment period under greenhouse conditions. Greenhouse lights were set to maintain a 16L : 8D photoperiod. Hobo water temperature loggers (Onset Computer Corp.) were placed in selected tanks to record temperature every 6 h.

Herbicide concentrations used for greenhouse evaluations are listed in Table 1. Treatments were static exposures, and the experiments were conducted for a period of 28 d. Supplemental water was added during the course of the study to replace water lost to evaporation. After the 28-d exposure period, shoot material was harvested and dried to a constant weight at 70 C for a minimum of 48 h.

Water samples were collected immediately after treatment, 7 DAT, and 28 DAT in selected tanks to determine exposure concentrations. Lack of potential for photolytic degradation has previously been demonstrated in studies conducted in these greenhouses (Netherland 2015). Each treatment was replicated three times, and each study was repeated.

Statistical Analysis. Equation 1 is the four-parameter log-logistic dose–response curve used to estimate EC_{50} for different measures of plant response. Estimation of this nonlinear regression model was performed using the drc package in R software (R 3.2.2, R Core Team 2015: https://www.R-project.org/). Methodology of this approach is described in detail by Knezevic et al. (2007) and Ritz and Streibig (2005):

$$Y = c+(d-c)/\{1+exp[b(\log x-\log e)]\} \quad [1]$$

The parameters b, c, d, and e estimate the relative slope at e, lower limit of Y, upper limit of Y, and midpoint of Y, respectively. The three-parameter form of Equation 1 ($c = 0$) was used when it was logical to restrict the lower limit to 0. The dependent variable Y consists of treatment averages ($n = 3$ or 4) within replicate studies ($n = 2$) for dry weight or for inhibition indices that relate response relative to the control calculated using dry weight, fresh weight, and plant length. The EC_{50} was estimated as the dose rate (x) corresponding to the midpoint (e) between the lower (c) and upper limit (d) for dry weight or the dose rate corresponding to 50% inhibition of specific growth rate or 50% inhibition in yield. Estimates of EC_{50} were compared for SX-1552 and SX1552A using the selectivity index (Ritz and Streibig 2005).

Final dry weight was estimated directly using model 1 as recommended by Knezevic et al. (2007). Graphical comparisons were performed by converting predicted values and sample means to percent dry weight reduction relative to the control. Model predictions were converted using the predicted upper limit (d) as the predicted control level and using the sample mean control (rate = 0) average for sample means.

Measures relative to the control were defined by specific study protocols as percent inhibition of specific growth rate (%Ir in Equation 2) and percent inhibition in yield (%Iy in Equation 3):

$$Ir = 100x(\mu_c-\mu_t)/\mu_c \quad [2]$$

Specific growth rate in Equation 2 was calculated for control (μ_c) or treated (μ_t) as the natural log of the

final divided by initial mean values divided by days (ln[final/initial]/days) for each replicate study. Equation 2 was modified when final size was less than initial size because this is when treatment-specific growth rates (μ_t) estimate necrosis/mortality on the basis of initial size rather than growth. Without modification, this results in no upper limit on %*Ir* and contradicts the log-logistic modeling approach used here. The focus on growth inhibition was maintained by restricting maximum %*Ir* to 100% (setting $\mu_t = 0$) when final size was less than initial size.

$$Ir = 100x(b_c - b_t)/b_c \quad [3]$$

Mean growth (b) in Equation 3 was calculated for control (b_c) or treated (b_t) as the average final minus average initial for each replicate study. Inhibition of yield (%*Iy*) can exceed 100% when treatment growth is negative.

A Dunnett's test ($\alpha = 0.05$) comparing dry weight biomass of treated vs. nontreated plants was performed to determine a lowest observed effect concentration (LOEC) across the broad range of SX1552 concentrations tested.

Results and Discussion

Growth Chamber Trials. In 14-d assays, reference plant biomass increased by 2.8 to 5.1 times the initial biomass for the different test species. OECD guidelines require that doubling of biomass and mean coefficient of variation between reference plants be less than 35% (OECD 2014). Both of these requirements were met in all of our growth chamber studies. All nontreated control plants were robust and actively growing throughout the trials and at the time of harvest. Water sampling after treatments with the SX-1552 formulation at 1 DAT indicated that 41 to 56% of applied SX-1552 had remained in the parent form, whereas the rest had converted to SX-1552A. Results from water sampling at 7 and 14 d indicated that SX-1552 had fully converted to SX-1552A, with recoveries at 7 and 14 d ranging from 89 to 112% of nominal treatment concentrations. Samples collected at 1 and 14 DAT with SX-1552A resulted in recoveries ranging from 94 to 108% of nominal concentrations. Results of this water sampling confirmed that target concentrations were achieved.

EWM was sensitive to both SX-1552 and SX-1552A, with EC_{50} values of 0.11 and 0.23 μg ai L^{-1} (Table 2, Figure 1). For both formulations, the LOEC value was 0.1 μg ai L^{-1}. Symptom development was rapid with characteristic auxin-like epinasty of the apical shoots noticed within 1 d of treatment. Megalodonta sensitivity to SX-1552 and SX-1552A resulted in EC_{50} values of 11.3 and 14.5 μg ai L^{-1} respectively (Table 2, Figure 1). LOEC values of 3 and 9 μg ai L^{-1} were determined for SX1552 and SX1552-A, respectively, whereas a concentration of 81 μg ai L^{-1} reduced biomass by greater than 90%. The visual auxin symptoms were greatly reduced for megalodonta compared with EWM.

Elodea sensitivity to SX-1552 and SX-1552A yielded EC_{50} values of 6.9 and 13.1 μg ai L^{-1} respectively, with both forms yielding a LOEC value of 9 μg ai L^{-1} (Table 2, Figure 1) The EC_{50} values indicated a difference between SX-1552 and SX-1552-A, (Table 2). There was no viable biomass for harvest at the highest concentration evaluated in this trial (81 μg ai L^{-1}). Slight visual auxin-like symptoms were noted on this monocot at the higher concentrations; however, the primary symptom noted was necrosis along the length of the stems. Hydrilla was much more sensitive, with EC_{50} values of 1.4 μg ai L^{-1} (SX-1552) and 2.5 μg ai L^{-1} (SX-1552-A) and a LOEC of 1 μg ai L^{-1} (Table 2, Figure 1). A difference in the EC_{50} value for SX-1552 and SX-1552-A was also noted for hydrilla. There was very limited biomass for harvest at concentrations > 9 μg ai L^{-1}. In addition to auxin-like symptoms at the shoot tips, this monocot became brittle and shoots readily separated upon slight disturbance in the first day or two

Table 2. Final dry weight (g) 50% effective concentration (EC_{50}) comparisons (standard error) for Eurasian watermilfoil (EWM), megalodonta (MEG), elodea (ELO), Hydrilla (HYD), and crested floating heart (CFH) after exposure to SX-1552 and SX-1552A.

Study type	Formulation	EWM	MEG	ELO	HYD	CFH
		EC_{50} (e)[a]				
Growth chamber	SX-1552	0.11 b (0.11)	11.3 a (2.0)	6.9 b (0.6)	1.4 b (0.1)	5.6 b (0.6)
	SX-1552A	0.23 ab (0.33)	14.5 a (2.8)	13.1 a (1.0)	2.5 a (0.3)	23.9 a (4.0)
Mesocosm	SX-1552	0.12 b (0.01)	6.1 b (0.2)	—	—	—
	SX-1552A	0.58 a (0.04)	—	—	—	—

[a] EC_{50} (μg ai L^{-1}) values with the same lowercase letter within a species are not significantly different at the 5% level.

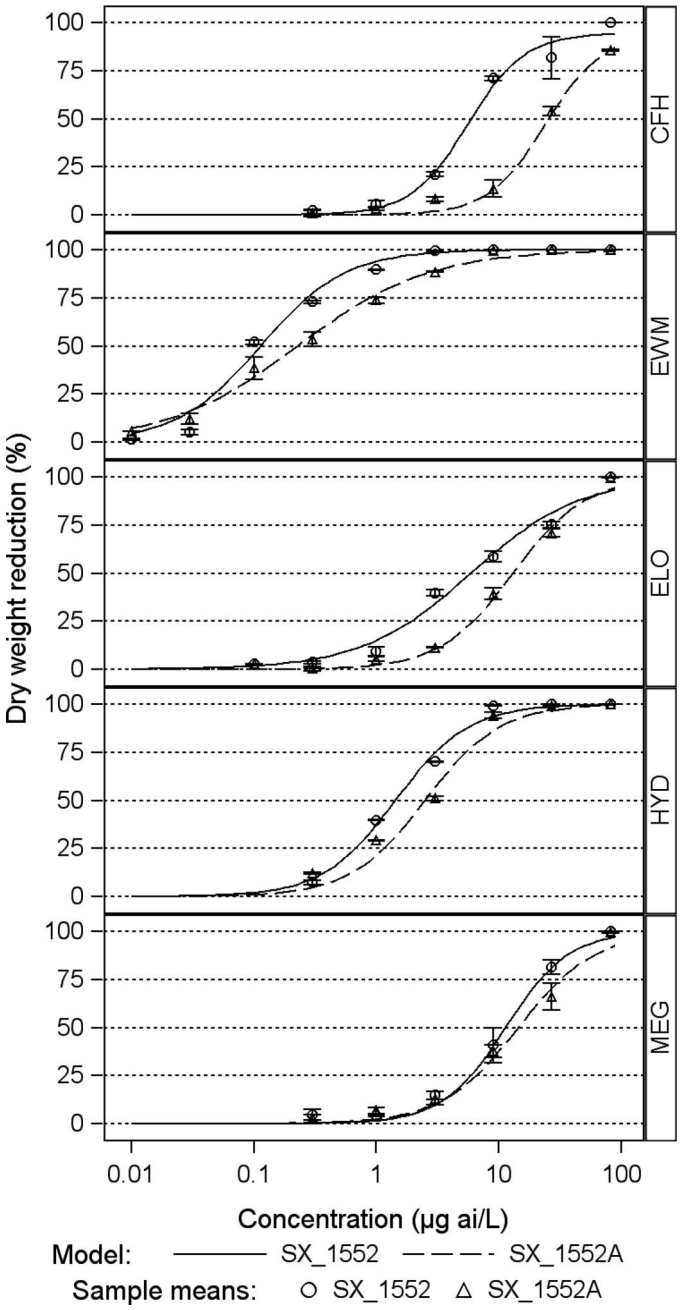

Figure 1. Logistic regression was used to plot dry-weight biomass reduction for five aquatic plant species after exposure to SX1552 (ester) and SX1552A (acid). Each symbol represents the mean value (± standard error, $n = 4$). Abbreviations: CFH, crested floating heart; EWM, Eurasian watermilfoil; ELO, elodea; HYD, hydrilla; MEG, megalodonta.

posttreatment. At harvest, plants that had been treated at concentrations > 3 µg ai L^{-1} had waterlogged stems (aerenchyma tissue that is normally filled with air was full of water) and the limited amount of remaining tissue lacked integrity.

CFH also showed differential sensitivity to SX-1552 and SX-1552A, with EC_{50} values of 5.6 and 23.9 µg ai L^{-1} respectively (Table 2, Figure 1). The LOEC value for the formulation was 3 µg ai L^{-1}, whereas the SX-1552-A value was 9 µg ai L^{-1}. CFH displayed a rapid onset of visual symptoms with notable stem elongation within 1 d after exposure to concentrations from 1 to 3 µg ai L^{-1}. Although these initial symptoms were easy to distinguish, they did not translate to impacts on biomass at the lower treatment concentrations. There was some chlorosis noted on surface leaves within 5 to 10 DAT. A clear visual difference between the activity of SX-1552 and SX-1552-A was noted for this floating leaf plant.

Per the OECD protocol, EC_{50} values were also determined for several growth-based parameters. The three-parameter version ($c = 0$) of Equation 1 (parameter estimates not shown) was used to estimate percent inhibition of growth rate (*Ir*) and percent inhibition in yield (*Iy*). Estimates of EC_{50} are compared by formulation in terms of shoot length, fresh weight, and dry weight by species (Table 3). These data indicate some variation in predicted EC_{50} values for SX1552 against the different plant species. Specifically, higher EC_{50} values for the growth rate (*Ir*) data for elodea and CFH was noted. Nonetheless, most growth-based values were generally similar to the EC_{50} values determined on the basis of dry weights (Tables 2 and 3). Per the OECD guidelines, it is stated that "EC_{50} values calculated when using the % inhibition of yield (*Iy*) and average specific growth rate (*Ir*) are not comparable and this difference is recognized when using the results of the test." Overall, these analyses are being conducted on data that show consistent relationships within a species (e.g., dry weight vs. fresh-weight ratios or stem length vs. fresh weight). As such, the EC_{50} values were in general agreement regarding the sensitivity of each species to SX-1552 and SX-1552A.

Mesocosm Trials. Water temperatures ranged from 17.6 to 23.2 C during the course of mesocosm trials. During the 28-d pretreatment growth period, EWM biomass increased by a factor of 37.5 compared with initial shoot weights, and megalodonta increased by a factor of 18.4. During the 28-d study period, biomass of EWM increased by a factor 2.7 and megalodonta increased by a factor of 2.2. The combination of rapid growth rates and limited space eventually resulted in plants nearing or reaching carrying capacity and slowing growth rates in these tanks. All nontreated plants were robust and actively growing at the time of treatment and harvest. Results from water sampling at 7 and 28 DAT indicate that measured

Table 3. Estimation of 50% effective concentration (EC_{50}) (μg ai L^{-1}) as the dose that corresponds to 50% inhibition of growth rate (*Ir*) or inhibition in yield (*Iy*) in growth chamber (GC) and mesocosm (Meso) trials. EC_{50} (standard error) values within species followed by the same lowercase letter are not significantly different at the 5% level.

Study type	Form	Shoot length		Fresh weight		Dry weight	
		%*Ir*	%*Iy*	%*Ir*	%*Iy*	%*Ir*	%*Iy*
				Eurasian watermilfoil			
GC	SX-1552	0.15b (0.01)	0.10b (0.01)	0.17b (0.01)	0.10b (0.01)	0.16c (0.01)	0.10c (0.01)
	SX-1552A	0.35a (0.03)	0.19a (0.02)	0.41a (0.04)	0.17a (0.02)	0.39b (0.04)	0.17b (0.02)
Meso	SX-1552	—	—	—	—	0.12d (0.01)	0.09c (0.01)
	SX-1552A	—	—	—	—	0.68a (0.06)	0.38a (0.03)
				Megalodonta			
GC	SX-1552	3.6b (0.4)	3.0b (0.5)	9.1 (0.9)	6.9a (0.7)	8.9a (1.0)	7.0a (0.8)
	SX-1552A	7.3a (0.6)	6.0a (0.8)	10.8a (1.0)	9.1a (1.0)	10.9a (1.8)	8.7a (2.7)
Meso	SX-1552	—	—	—	—	6.4b (0.7)	4.7a (1.0)
				Elodea			
GC	SX-1552	3.0b (0.2)	2.8b (0.5)	26.2a (18)	7.1a (2)	21.0a (12)	6.3a (1)
	SX-1552A	7.4a (0.7)	6.8a (1.2)	34.1a (47)	13.0a (3)	28.3a (11)	12.2a (2)
				Hydrilla			
GC	SX-1552	1.7b (0.2)	1.1b (0.1)	2.0b (0.2)	1.1b (0.1)	2.1b (0.2)	1.2b (0.1)
	SX-1552A	3.4a (0.4)	1.8a (0.2)	3.4a (0.2)	1.9a (0.2)	3.6a (0.3)	1.8a (0.2)
				Crested floating heart			
GC	SX-1552	5.9b (0.3)	5.4b (0.5)	7.0a (0.2)	4.9a (0.3)	7.2a (0.9)	5.0b (0.5)
	SX-1552A	26.6a (2.5)	17.6a (2.5)	41.1a (27)	26.1a (35)	33.2a (18)	21.0a (4)

concentrations of SX-1552 and SX-1552A were 87% ± 5% of the target concentrations.

EWM was sensitive to both SX-1552 and SX-1552A in larger-scale mesocosms under greenhouse conditions. Despite the larger initial size and more robust plants, EC_{50} values for SX-1552 and SX-1552A were 0.12 and 0.58 μg ai L^{-1} respectively. (Table 2). LOEC values were 0.1 and 0.3 μg ai L^{-1} for SX-1552 and SX-1552A. Within 1 to 2 d after exposure, plants became very brittle and stems fragmented into small pieces after slight disturbance. Comparison of growth chamber and mesocosm data suggests that despite different initial plant biomass and study conditions, EWM responded in a similar manner (Table 2, Figure 2). Megalodonta susceptibility in the mesocosm trials was generally similar to results observed in the growth chamber trials. The EC_{50} value for SX-1552 was 6.1 μg ai L^{-1}, whereas the LOEC was 9 (Table 2). Given the broad rate structure evaluated, there were minimal impacts on plant growth at 3 μg aiL^{-1}, whereas the 9 μg ai L^{-1} treatment resulted in > 65% biomass reduction. The EC_{50} value calculated for megalodonta was significantly lower for the greenhouse vs. the growth chamber trials (6.1 vs. 11.3 μg ai L^{-1}). It is possible that improved growth conditions in the mesocosms could explain the increased susceptibility of the megalodonta when compared with the space limitations observed in the 2-L beakers.

Results suggest that EWM is highly susceptible to both SX-1552 and SX-1552A. The EWM growth chamber and mesocosm trials were complementary and indicate that the EC_{50} values are well below

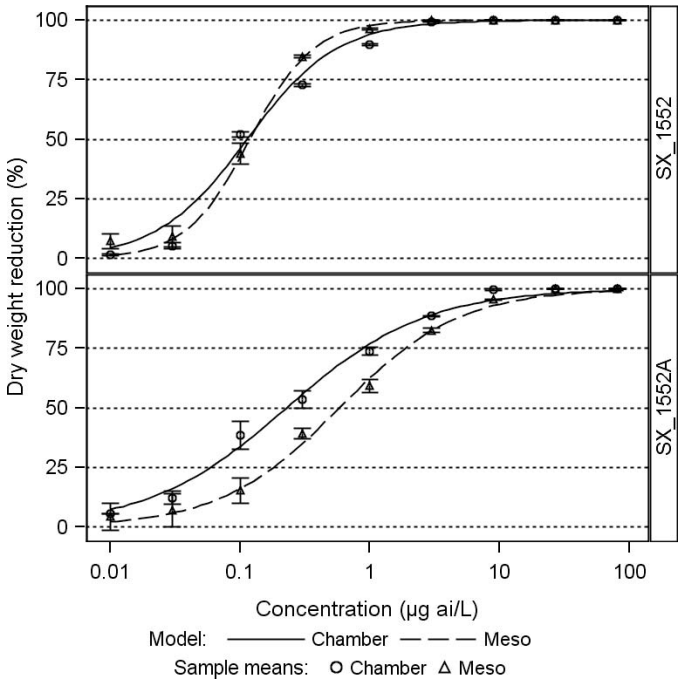

Figure 2. Logistic regression was used to plot dry-weight biomass reduction of Eurasian watermilfoil after exposure to SX1552 and SX1552A after growth chamber (chamber) and mesocosm (Meso) studies. Each symbol represents the mean value (± standard error, $n = 4$ for growth chamber trials and $n = 3$ for mesocosm trials).

1 μg L^{-1}. Across all species, SX-1552 resulted in lower EC_{50} values vs. SX-1552A; however, because of the rate structure evaluated the LOEC was often similar between the forms. The EC_{50} value for megalodonta was 63 to 102 times greater than for EWM. Interestingly, a dichotomy was also observed for the two monocotyledons. The EC_{50} values for the native elodea species were 4.9 to 5.4 times greater than that for the invasive species hydrilla. Given the invasive nature of both EWM and hydrilla in the United States, this level of SX-1552 activity warrants further investigation for potential use against these species.

These trials were based on extended static exposures to SX-1552, and therefore the results need to be viewed in context, as static exposures can result in enhanced activity against a given submersed species in small-scale systems (Mohr et al. 2013). For example, mesocosm evaluation of static exposures (> 3 wk) of the auxin-mimic herbicides 2,4-D and triclopyr demonstrated high levels of activity for these herbicides on EWM at rates ranging from 25 to 75 μg ai L^{-1} (Glomski and Netherland 2010), yet typical use rates for these products range from 500 to 2,000 μg ai L^{-1}, as most treatments for submersed aquatic management are subject to rapid dispersion from the treatment site (Netherland 2015). The current results suggest that SX-1552 produces strong auxin-like symptoms, can result in rapid onset of injury and loss of EWM biomass, and is at least an order of magnitude more active on EWM when compared with products such as 2,4-D and triclopyr (Glomski and Netherland 2010; Green and Westerdahl 1990; Netherland and Getsinger 1992). Although 2,4-D and triclopyr can elicit symptoms on hydrilla at high concentrations, neither herbicide provides hydrilla control at maximum-labeled use rates in the range of 2,500 to 4,000 μg L^{-1}. In this study hydrilla lost tissue integrity at 3 μg ai L^{-1} and was completely controlled at a concentration of 9 μg ai L^{-1} after a 14-d static exposure period to SX1552.

In examining the potential utility for utilizing the OECD protocol to evaluate other herbicides or potential impacts on different plant species, there are several inherent strengths as well as a few caveats. The current results suggest that products like SX-1552 might be well suited to this screening method. However, slow-acting aquatic herbicides that target plant-specific enzymes such as fluridone (phytoene desaturase inhibitor [PDS]), penoxsulam (acetolactate synthase [ALS] inhibitor), and topramazone (hydroxyphenylpyruvate dioxygenase [HPPD] inhibitor) can require up to 2 to 4 mo to provide plant control (Netherland 2015). Use of a protocol that focuses on short-term changes in biomass and growth may not be optimal for predicting activity of slow-acting herbicides. Research using a water-only assay (e.g., recently sprouted tubers or apical shoot meristem growing in Hoagland's solution) has provided valuable data on short-term changes in pigments, growth inhibition, or impacts on root growth (Berger et al. 2015; Mohr et al. 2013; Netherland 2011, 2015). Additional testing using the OECD protocol on these slow-acting herbicides is recommended and extending the length of these trials to 28 d may provide additional data to separate between concentrations that are likely to provide growth regulation vs. those concentrations that are likely to kill the plant.

Fast-acting contact herbicides like diquat would demonstrate high levels of activity using this protocol, as EWM is very sensitive to this herbicide. Moreover, extended unrealistic exposures to diquat in these assays (due to lack of binding to suspended sediments or organic particulates in an assay) are not characteristic of field conditions. In this case, testing EWM would indicate that diquat is highly active for both regulatory and operational predictions; however, the impact of turbidity on diquat activity in the field would likely result in greatly reduced activity (Poovey and Getsinger 2002). Fast-acting products that require moderate exposure periods such as 2,4-D, triclopyr, endothall, and SX-1552 can be evaluated in a relatively short period of time and these products tend to perform in a similar manner under a broad range of environmental conditions (e.g., turbidity, pH, temperature, etc).

The growth chamber results with SX-1552 were validated at the mesocosm scale for the two dicot species tested. Such outcomes will likely vary for contact or systemic herbicides. Several submersed aquatic plants are highly susceptible to the rapid-acting protoporphyrinogen oxidase inhibitor flumioxazin under growth chamber conditions. Yet flumioxazin activity can be reduced under increasing pH as the molecule is rapidly hydrolyzed at a higher pH (Mudge and Haller 2006).

The OECD protocol offers a good model for screening inherent herbicide activity on submersed plants under relatively long-term exposures, but could easily overestimate risk when relying on a single species for risk assessment purposes. In this study, EWM was by far the most sensitive aquatic plant species to SX-1552. It could have also been the most tolerant, or shown no effect. Aquatic plant community interactions should be considered,

involving multiple species of submersed or floating species. For example, in this study, the desirable native aquatic plants were more tolerant than the invasive species EWM and hydrilla. In addition, the exposure scenario should be kept in perspective after a terrestrial application of SX1552. Exposures significantly less than 14 or 28 d would generally be expected. Additional small-scale tests of other submersed native and invasive dicots and monocots at the chamber scale are recommended. The ability to utilize results from studies conducted at this scale provides an efficient and cost-effective method to screen plants under a variety of concentrations and exposure scenarios common to treatment of aquatic sites.

Overall these study results confirm a high level of SX-1552 activity on several aquatic species and the greater activity of SX-1552 and SX-1552-A. For SX 1552 the growth chamber studies were predictive of mesocosm results. Although the OECD protocol is currently specific to EWM for regulatory purposes in Europe, the current results suggest that this protocol (or modified versions of this protocol) could be used for multiple herbicides or aquatic plant species. Predicting herbicide activity on rare or threatened species or using this protocol to better refine knowledge of invasive plant response to a given herbicide are two areas where this small-scale assay could provide information that would improve study design for large-scale mesocosm testing.

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