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Imazamox absorption, desorption, and metabolism by Eurasian watermilfoil

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ABSTRACT

Eurasian watermilfoil (*Myriophyllum spicatum*) is a submersed invasive species currently infesting 45 states, including Colorado. Several laboratory experiments were conducted to determine the response of Eurasian watermilfoil to imazamox [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid]. Experiments were (1) imazamox absorption rate using ^{14}C-imazamox, (2) the influence of external imazamox concentration on absorption, (3) imazamox desorption when plants were transferred to clean water, and (4) imazamox metabolism over a 6 d time course. Imazamox absorption by Eurasian watermilfoil 24 h after treatment (HAT) was only 0.5% of the herbicide applied, and absorption increased to 0.97% 72 HAT. External imazamox concentration affected imazamox absorption. At 200 µg L^{-1} imazamox, Eurasian watermilfoil plants absorbed 1.05 µg/plant, while at 800 µg L^{-1} absorption increased to 4.06 µg/plant. The percent of applied imazamox absorbed was the same regardless of the external concentration, indicating that absorption was the result of simple diffusion driven by a concentration gradient. Desorption occurred rapidly, reaching equilibrium 12 h after plants were transferred to clean water, with 46% of absorbed imazamox moving into the surrounding water column. The metabolism study indicated 69.04% of absorbed ^{14}C-imazamox was found in the bound fraction 144 HAT, while 11.52% appeared as soluble metabolites, and only 21.44% as intact imazamox.

Key words: absorption, imazamox, *Myriophyllum spicatum*, radiolabeled.

INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is a submersed aquatic macrophyte considered invasive across much of the United States. While the upper Midwest has some of the most significant infestations, heavy infestations can also be found in lakes along Colorado's Front Range as well as in irrigation canals. Eurasian watermilfoil can drastically impact recreation, aquatic vertebrate habitat, and the ability to efficiently deliver water.

Eurasian watermilfoil behaves as an herbaceous perennial. When waters warm in spring, single shoots will grow rapidly toward the surface. Once shoots reach the water surface, they branch profusely and form large, dense mats. After branching at the water surface, plants flower and fragment. Living fragments may fall to the bottom and form new plants, while other plants will resprout from the shoot crowns the following year. These shoot fragments then fall to the bottom of the water body, and the cycle starts over. Although Eurasian watermilfoil does produce viable seeds, the main method of spread and reproduction is through vegetative fragments (Smith and Barko 1990). Eurasian watermilfoil thrives in waters 1 to 4 m deep (Nichols and Shaw 1986), but with greater water clarity, it can grow from a depth of 10 m (Aiken et al. 1979). Maximum growth is achieved at 30 to 35 C, which also corresponds to the temperature range for maximum photosynthetic activity (Smith and Barko 1990).

Eurasian watermilfoil has several characteristics that contribute to its invasiveness. It often establishes early in the growing season when water temperatures are relatively low (Barko et al. 1982), thereby shading native plants. Because light is a major limiting factor in aquatic systems, this can make it difficult for native species to establish and can lead to dense monocultures of Eurasian watermilfoil. Also, colonizing through fragments allows Eurasian watermilfoil to be spread easily by animals, human activities, and flowing water.

Herbicides represent a more long-term management strategy for Eurasian watermilfoil. Contact herbicides labeled for aquatic use include endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid), diquat (6,7-dihydrodipryido[1,2-α:2',1'-c]pyrazinediium ion), and copper (Gettys et al. 2009). An advantage of using contact herbicides is that they may require a shorter contact time than systemic herbicides, but they may only provide temporary control. Systemic herbicides currently labeled for Eurasian watermilfoil control include 2,4-D ((2,4-dichlorophenoxy)acetic acid), triclopyr ([(3,5,6-trichloro-2-pryidinyl)oxy]acetic acid), and fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone) (Gettys et al. 2009). Systemic herbicides may provide more long-term control than contact herbicides, but require a longer exposure time to be effective.

Imazamox is a newly registered herbicide that inhibits acetolactate synthase (ALS), the first committed step in branched chain amino acid biosynthesis. Imazamox is effective on a variety of emergent and submersed species, including Eurasian watermilfoil. Imazamox also has a favorable environmental profile, which has led to a tolerance exemption from the US Environmental Protection Agency (EPA), as well as minimal irrigation restrictions on turf and crops.

Previously published research that has focused on pesticide absorption in aquatic plants presumed that aquatic plants are bioaccumulaors of highly lipophilic pesticides, including atrazine (log K_{ow} 2.34), linuron (log K_{ow} 3.00), and di-

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azinon (log K_{ow} 3.81; Crum et al. 1999, de Carvalho et al. 2007). The most lipophilic herbicide currently labeled for aquatic use is fluridone (log K_{ow} 1.87). Little information is available regarding the absorption (bioaccumulation) of highly water-soluble compounds such as 2,4-D (log K_{ow} 0.18) and triclopyr (log K_{ow} -0.44). Imazamox more closely resembles these water-soluble herbicides with a log K_{ow} of 0.73; therefore, previous studies with highly lipophilic pesticides do not accurately reflect absorption for a highly water-soluble compound such as imazamox. A lipophilic compound would likely accumulate in plant tissue, but for a more water-soluble compound, absorption driven by a concentration gradient between the water column and water in the plant may be the main route of uptake.

Currently no information is available regarding the behavior of imazamox in aquatic plants; therefore, the objectives of this project were (1) to examine imazamox absorption and desorption; (2) to determine the effect of imazamox concentration in the water column on absorption; and (3) to determine the rate of imazamox metabolism by Eurasian watermilfoil.

MATERIALS AND METHODS

Eurasian watermilfoil shoot fragments were collected from a single population in the Leggett Ditch north of Boulder, CO (4013' N, 10508' W) in Fall 2006. The fragments were then cut into 15 cm pieces and the distal end was planted in 5 cm dia × 10 cm deep plastic cups filled with fine sand. Each pot was fertilized with 0.5 g of slow release fertilizer (Osmocote Classic 14-14-14, The Scotts Company, USA) at planting to maintain active growth. Plants were grown in tap water in a 1.2 by 2.4 by 0.3 m fiberglass tank in the greenhouse until they produced roots. The photoperiod was 10:14 h light:dark cycle with natural light supplemented with 400-watt sodium halide light bulbs. Temperature in the greenhouse was set at a 24 C during the day and 18 C at night. Plants that grew too large for use in laboratory experiments were recycled by removing the apical 15 cm of each plant and replanting as previously described. Unless otherwise noted, potted plants for laboratory experiments were removed from the fiberglass tank and placed in 1.2 L glass cylinders and submersed in 1 L of tap water. After transferring the plants to cylinders, they were allowed to equilibrate in a growth chamber for 24 h prior to treatment with ^{14}C imazamox. Following treatment, the cylinders and plants were moved to a growth chamber with a 10:14 h light:dark cycle and temperature set at 20 C during the light period and 10 C during the dark period, with a light intensity during the light period of 250 moles m^{-2} s^{-1}.

Unless otherwise noted, for all experiments plants were harvested, divided into aboveground and belowground parts, dried to a constant weight at 60 C for 24 h, and absorbed ^{14}C was determined by biological oxidation (OX500, R. J. Harvey Instrument Co., USA) with 10 mL of ^{14}C trapping cocktail (OX-161, R. J. Harvey Instrument Co., USA). To confirm the amount of ^{14}C-imazamox present in the treatment solutions, 100 µL water samples were collected using a pipette and transferred to 20 mL scintillation vials. Scintillation cocktail (10 mL) was then added to each vial (6013371, Ultima Gold LLT, PerkinElmer, USA). Radioactivity for both plant and water samples was then quantified using a liquid scintillation spectroscopy (LSS; Packard 2500R, PerkinElmer, USA).

Imazamox Absorption Rate

Fifteen rooted plants were treated with 200 µg L^{-1} imazamox that contained 21.7 KBq of ^{14}C imazamox (specific activity 1850 KBq mg^{-1}). The plants were harvested at 6, 12, 24, 48, and 72 h after treatment (HAT). Three plants were harvested at each time point, and samples were analyzed as described above. Three plants were randomly selected for harvest at each time point, each plant representing one replication. The study was repeated.

Influence of External Concentration on Imazamox Absorption

Once placed in 9 glass cylinders, rooted plants received 1 of 3 treatments: (1) 200 µg L^{-1} plus 16.7 KBq; (2) 400 µg L^{-1} plus 33.3 KBq; or (3) 800 µg L^{-1} plus 66.7 KBq of formulated imazamox plus ^{14}C imazamox, respectively. Three plants were treated at each concentration harvested 24 HAT and analyzed for ^{14}C as described above. The study was repeated.

Imazamox Desorption

To determine imazamox desorption rate, 3 rooted plants were first treated with 800 ng L^{-1} imazamox concentration that contained 216.7 KBq of ^{14}C imazamox. Plants were allowed to absorb imazamox for 24 h then triple rinsed in clean water and placed in jars that contained 50 mL of tap water. The amount of imazamox desorbing from treated plants was determined by taking 1 mL water samples at 0, 1, 2, 4, 6, 12, 24, 48, and 72 HAT, and radioactivity was determined using LSS. After 72 h in the clean water, whole plants were harvested, dried, and oxidized to determine the amount of ^{14}C remaining in the plant. There were three replicate water samples taken per time point. The study was repeated.

Imazamox Metabolism

Plants were placed in 250 mL jars containing 200 mL of water and an 800 ng/mL imazamox concentration that contained 90 KBq of ^{14}C imazamox. Plants were then harvested at 24, 48, 72 and 144 HAT. Shoot material was placed in 50 mL test tubes with 10 mL of an acetone:water (9:1 v/v) solution. Tissue was ground using a mechanical tissue homogenizer (302968, Tempest, VirTis, USA) and the homogenate was transferred to 50 mL centrifuge tubes with 0.45 µm filter inserts (6831-0409, VectraSpin 20, Whatman, England). Samples were centrifuged for 15 min at 3000 RPM. Next, the filter was rinsed using 2 mL of the acetone:water solution and then centrifuged for 5 min at 3000 RPM. This was repeated twice and the filtrate was transferred to clean 50 mL glass centrifuge tubes. Samples were then concentrated using a sample evaporator (Rapidvap, Labconco Corp., USA) until most of the acetone was removed. The remaining liquid was then transferred to 2 mL centrifuge tubes with 0.45 µm filter inserts (Costar Spin-x 8170, Corning Inc., USA) and centrifuged for 10 min. The filtrate was then removed and placed in 0.4 mL inserts, and ^{14}C imazamox was de-

termined with reverse phase high performance liquid chromatography (HPLC) using a C8, 2.1 × 150 mm column (Zorbax, USA). The injection volume was 100 µL. Imazamox eluted at 14 min using the following gradient: 89.95% water:10% acetonitrile:0.05% phosphoric acid solution to a 69.95% water:30% acetonitrile:0.05% phosphoric acid solution over 25 min with a flow rate of 0.3 mL/min. Radioactivity was quantified using an inline radioactive detector (-Ram Radioactivity Detector Model 2B, IN/US, USA). All material retained by the centrifuge filters was dried and oxidized as previously described. Three replicates (plants) were harvested at each time point. The study was repeated.

Data Analysis

Levene's test for homogeneity of variance was conducted using JMP (Version 7.0.1, SAS Institute, 2007) to determine if data from repeated experiments could be combined. Based on results of Levene's test for homogeneity of variance, data from repeated experiments were combined for statistical analyses for all studies. All data presented represents mean values, and errors are presented as standard error. Regression analyses were performed and data plotted using SigmaPlot (Version 9, SYSTAT, 2005).

RESULTS AND DISCUSSION

Imazamox Absorption Rate

Imazamox absorption over a 72 h time course was low compared to the amount applied (Figure 1). The function that best described imazamox absorption by Eurasian watermilfoil was

$$y = \frac{a}{1 + e^{\left(\frac{-(x - x_0)}{b}\right)}} \qquad [1]$$

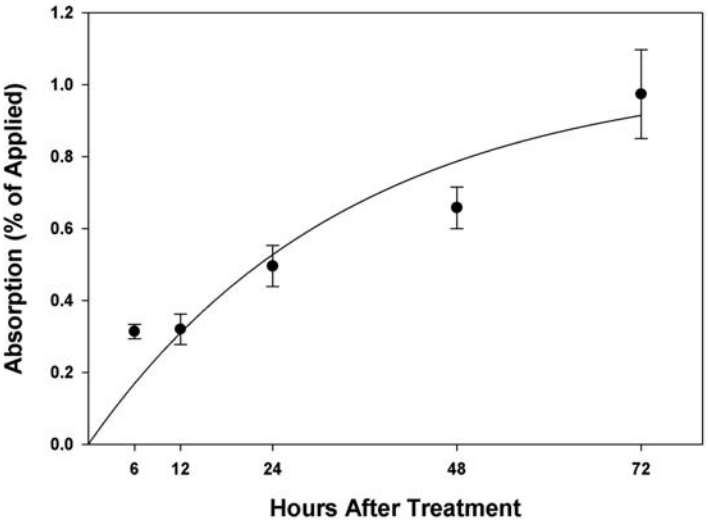

Figure 1. Imazamox absorbed over a 72 h time course, expressed as a percentage of total applied ^{14}C-Imazamox. Regression line represents the function y = 1.51/(1+*e*^((-(x-52.37))/33.93)), (r^2 = 0.903).

where a = 1.51, b = 33.93, and x_0 = 52.37. Only 0.5% ± 0.06 of applied imazamox was absorbed in the first 24 HAT, and by 72 HAT the maximum amount absorbed was 0.97% 0.12. These results indicate that 50% of the imazamox absorption occurs in the first 24 HAT and the remaining 50% occurs over the next 48 HAT. This is in sharp contrast to terrestrial species like jointed goatgrass (*Aegilops cylindrical* Host) and feral rye (*Secale cereale* L.), which absorbed 58 and 44% of applied imazamox by 24 HAT, respectively (Pester et al. 2001). Low imazamox absorption by Eurasian watermilfoil was very similar to herbicide absorption in other submersed macrophytes. Sago pondweed (*Stuckinea pectinatus* [L.] Böerner) and Richardson pondweed (*Potamogeton richardsonii* [Benn.] Rydb.) absorbed only 0.4 and 0.7% of applied fluridone at the end of a 14 d time course, respectively. Due to the high water solubility and low Log K_{ow} of imazamox, we expected the concentration of imazamox in the plant to be nearly equal to the external concentration, but the actual concentration inside of the plant was 6.93 times the external concentration 72 HAT, based on total radioactivity. De Carvalho et al. (2007) suggested that more lipophilic compounds would easily permeate membranes, while more water soluble compounds may be absorbed by acid trapping. We predict that the likelihood of this happening with a water-soluble compound like imazamox would be less likely when macrophytes such as Eurasian watermilfoil are present. Photosynthesizing aquatic plants can significantly increase water pH, working against this acid trapping hypothesis (Sculthorpe 1967). Of the previous work, imazamox would be most similar to the uptake of 3,5-D by *Lagarosiphon major* (Ridley) Moss, which showed decreased absorption as pH increased. The dissociated form of 3,5-D has a log K_{ow} of 0.25 and more accurately represents the same trends in absorption demonstrated by our research with imazamox (de Carvalho 2007).

Influence of External Concentration on Imazamox Absorption

Imazamox absorption was strongly correlated with external herbicide concentrations over a range of 200-800 µg L^{-1} (Figure 2). This relationship appears to be linear with a corresponding function of

$$y = y_0 + a * x \qquad [2]$$

where y_0 = 0.015 and a = 0.005. When treatment concentration increased from 200 to 400 µg L^{-1}, the amount of imazamox absorbed increased from 1.05 to 1.99 g/plant, and when the concentration increased from 400 to 800 µg L^{-1}, the amount of imazamox absorbed on a whole plant basis increased from 1.99 to 4.06 µg/plant. The amount of imazamox absorbed was approximately 0.5% of the amount applied. The direct linear relationship between external concentration and the amount of imazamox absorbed indicates that absorption was driven by the concentration gradient between the water column and plant. Eurasian watermilfoil possesses a very thin cuticle, which offers little resistance to diffusion into plant tissue (Sculthorpe 1967). Given imazamox's high water solubility (4413 mg L^{-1}; EPA 1997) and Eurasian watermilfoil's greatly reduced cuticle, it seems likely a water soluble compound such as imazamox would easily

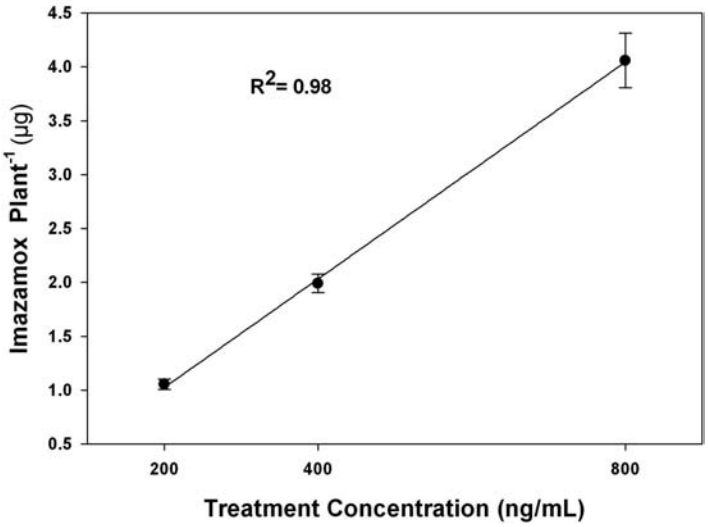

Figure 2. Total amount of imazamox absorbed per plant at treatment concentrations of 200, 400, and 800 µg L^{-1} following a 24 h exposure period. Regression line represents the function y = 0.015 + 0.005*x, (r^2 = 0.984).

diffuse into plant tissue and partition into water-filled free space, eventually coming to equilibrium with the surrounding water column if the plants were exposed over a longer period.

There is little evidence of significant translocation from the shoot to root tissue. The shoot accounted for approximately 98% of absorbed imazamox, while the root accounted for only 2% (Table 1). This partitioning in shoot and root biomass remained consistent across all three treatment concentrations, indicating little or no translocation to roots. This lack of basipetal translocation has also been observed in Sago pondweed and Richardson's pondweed when shoots were treated with fluridone (Marquis et al. 1981). While this appears to hold true in other aquatic species, it is a sharp contrast to that seen for imazamox in terrestrial species. Pester et al. (2001) found that 96 HAT, 27 and 20% of absorbed imazamox had translocated to the roots in feral rye and jointed goatgrass, respectively. So, even though imazamox is readily translocated in terrestrial species, its behavior in Eurasian watermilfoil is similar to results previously published for fluridone in Richardson's pondweed and Sago pondweed.

Imazamox Desorption

Imazamox was rapidly desorbed when treated plants were transferred to tap water with no herbicide. The amount desorbed was determined as a percentage of total imazamox absorbed on a whole plant basis (Figure 3). Imazamox desorption can be described by the function

$$y = a(1\text{-}e^{(-bx)}) \qquad [3]$$

where a = 46.188 and b = 0.905. In the first 12 HAT, 46% of absorbed imazamox moved out of the plant and into the surrounding water column. Imazamox readily moved out of the plant and eventually reached equilibrium with the surrounding water column by the end of the 72 h time course. We did not continue the desorption process by continually exposing plants to clean water, so there is no way to determine if some portion of the radioactivity remaining in the plant was bound and not easily desorbed. These data do support the theory that imazamox absorption and desorption are driven mainly by a concentration gradient, and that a dynamic equilibrium is established between the water column and aquatic vegetation. Our observed rapid photolysis of imazamox in whole lake treatments with a half-life of ~10 d (data not shown) would suggest that the maximum concentration in the plant will occur soon after application and will decline primarily due to decreasing external concentrations.

Table 1. Partitioning of imazamox into aboveground and belowground biomass expressed as a percent of total absorbed ^{14}C-imazamox following a 24 h exposure time.

Treatment Concentration	% in Shoot	% in Root	Standard Error
200 ng/mL	97.99	2.01	0.75
400 ng/mL	97.79	2.21	0.93
800 ng/mL	97.56	2.44	0.64

Imazamox Metabolism

Imazamox metabolism was determined by dividing radioactive fractions into 3 categories: intact imazamox, soluble metabolites, and bound metabolites (Figure 4). No attempt was made to identify metabolites. Intact imazamox was identified as the radioactive peaks corresponding to retention time of the imazamox standard. Predicted imazamox metabolism rates can be described by the power function

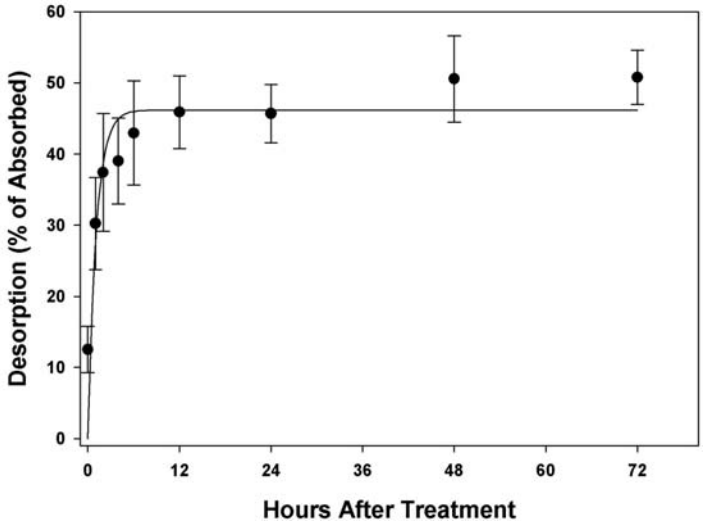

Figure 3. Desorption of ^{14}C expressed as a percentage of total absorbed ^{14}C following a 24 h treatment to 800 µg L^{-1} imazamox. Only total ^{14}C was measured and likely represents remaining imazamox and soluble metabolites. Regression line indicates predicted values as calculated using the function y = 46.188*(1-*e*^(-0.905*x)), (r^2 = 0.902).

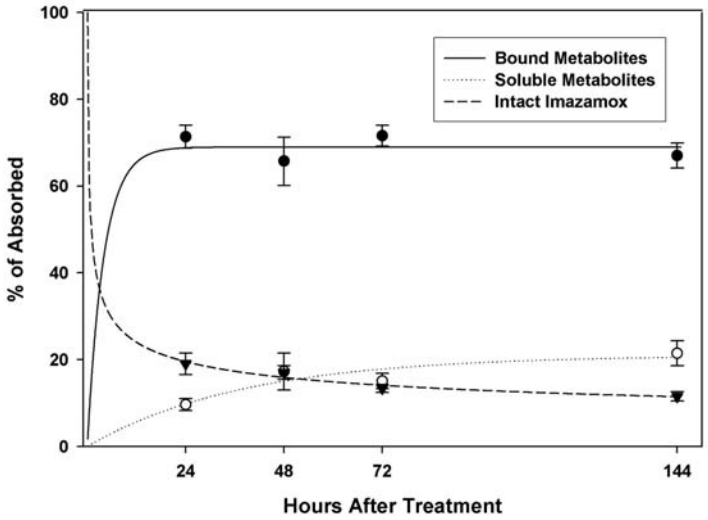

Figure 4. Imazamox metabolism expressed as a percentage of total absorbed imazamox separated into 3 fractions: (1) Intact Imazamox (y = 50.347*x^-0.298, r^2 = 0.997); (2) Soluble Metabolites (y = 21.0121*(1-*e*^(-0.026*x)), r^2 = 0.816); and (3) Bound Metabolites (y = 68.948*(1-*e*^(-0.249*x)), r^2 = 0.992).

$$y = ax^b \qquad [4]$$

where a = 50.347 and b = -0.298.

Other radioactive peaks that did not correspond to the retention time of the standard were considered soluble metabolites. This fraction can be described by the following exponential rise to max function to obtain predicted values:

$$y = a(1\text{-}e^{(-bx)}) \qquad ([5]$$

where a = 21.021 and b = 0.026. Bound metabolites were determined by oxidizing the remaining dried plant material following extraction and were assumed to be bound to plant tissue. This fraction was then estimated using equation 5 when a = 68.948 and b = 0.249.

Approximately 70% of the absorbed imazamox was found in the bound fraction 24 HAT, while 10% seemed to be soluble metabolites. Only 19% ± 2.47 remained as intact imazamox. The percent of absorbed radioactivity found in the bound fraction remained constant from 24 to 144 HAT. Over the same time period, the soluble metabolites increased to 21.44% ± 2.88 by 144 HAT, while intact imazamox decreased to 11.52% ± 1.02. Imazamox metabolism seems to occur rapidly in Eurasian watermilfoil compared to jointed goatgrass and feral rye. In these terrestrial species 75% of the imazamox remained intact 24 HAT, and even at 96 HAT, 25 to 50% remained intact. Based on predicted values, the half-life of imazamox in Eurasian watermilfoil was short (7.65 h) compared to feral rye (42 h) or jointed goatgrass (84 h; Pester et al. 2001). Considering the internal concentration found in the absorption study that was 6.93 times the external concentration, and percentage of that remained as intact imazamox 72 HAT (13.35%), the concentration of imazamox inside the plant was nearly equal to the external concentration. This provides additional support that imazamox absorption is driven by a concentration gradient. It seems that a significant amount of absorbed imazamox is quickly bound to plant tissue within 24 HAT and this fraction remains steady at around 70%. At later time points the remaining intact imazamox slowly decreases, while the amount of soluble metabolites slowly increases. These bound residues could be conjugated to lignins, or cell wall constituents. While bound metabolites are probably not phytotoxic, there is evidence from terrestrial species that hydroxylated metabolites of many imidazolinones remain phytotoxic but do not translocate (Shaner and Mallipudi 1991). In aquatic applications where the entire aboveground portion of the plant is exposed to the herbicide at one time, translocation may be less important.

Our field studies evaluating Eurasian watermilfoil control show that imazamox can provide multiple-season control at concentrations of 100 to 200 µg L^{-1} in whole lake treatments (data not shown). It seems that rapid imazamox absorption does occur, and absorption is driven by a concentration gradient. Although absorption driven by a concentration gradient allows for relatively fast absorption, this can also be a disadvantage in a system where imazamox concentration in the water column may drop quickly. If the external concentration were to drop, the herbicide seems to quickly diffuse out of the plant. Imazamox metabolism also occurs rapidly, with only about 20% of imazamox remaining intact by 24 HAT. Maintaining a treatment concentration can be difficult in flowing water and may not allow for sufficient absorption, and herbicide diffusion out of the plant may not provide adequate exposure time for control. Ongoing research is investigating optimal imazamox concentration, exposure time, and application timing for the maximum efficacy on Eurasian watermilfoil.

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