J. Aquat. Plant Manage. 45: 52-54

NOTES

Toxicity of Diquat and Endothall to Eastern Spiny Softshell Turtles (*Apalone spinifera spinifera*)

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INTRODUCTION

Populations of hatchling eastern spiny softshell turtles (*Apalone spinifera spinifera*) have shown a decline in Sodus Bay of Lake Ontario, Wayne County, New York since 1988 (Jerry Czech, unpublished monitoring data). Some people have associated this decline with the use of the aquatic herbicides diquat (6,7-dihydrodipyrido[1,2-*a*:2',1'-*c*]pyrazinediium) and endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid). Diquat and endothall are contact herbicides that are used to control Eurasian watermilfoil (*Myriophyllum spicatum* L.) and curly-leaf pondweed (*Potamogeton crispus* L.).

Information on the toxicity of these herbicides to turtles is generally not available in the literature. Toxicity tests for reptiles are not required for the registration of pesticides (40 CFR Part 158 2004), and there is limited data collected regarding the impact of aquatic herbicides on reptiles. Aquatic turtles, especially softshell turtles of the genus *Apalone* spp., may be particularly sensitive to pollutants due to their specific adaptations to aquatic habitats. Softshell turtles have skin with a greater level of water exchange compared to other reptiles (Dunson 1960). They also flush water into and out of their mouths and cloacas to obtain oxygen from the water. These characteristics provide possible routes of exposure that are not typically found in other reptiles.

The limited toxicity studies conducted with reptiles rely on "dosing" the test animals with a particular amount of toxicant, monitoring the animals over time, and calculating a median lethal (e.g., LD50) or no effect dose (NED). These are most commonly expressed in terms of mg of active ingredient per kg of body weight of the test animal. Due to the aquatic nature of the softshell turtle, our study examines the toxicity of diquat and potassium endothall in a manner that is akin to fish toxicity testing. We exposed our test turtles to a range of herbicide concentrations in the water and monitored the test animals over time. The results are expressed as lethal or no effect concentrations rather than doses, which allow easier comparison to concentrations of chemicals in the aquatic environment.

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MATERIALS AND METHODS

The herbicides used in all of the toxicity tests were commercial liquid formulations registered for use in the USA and New York State. Reward (Syngenta Crop Protection, Inc. Greensboro, NC) is a formulation containing 240 g of diquat cation per liter. All diquat concentrations are expressed as mg/L as cation concentration. Aquathol K (Cerexagri, Inc., King of Prussia, PA) contains 507 g of potassium endothall per liter. All endothall concentrations are expressed as mg/L potassium endothall.

Softshell turtle hatchlings were from captive bred turtles held by the Cold Spring Harbor Fish Hatchery and Aquarium (Nassau County, NY). Softshell turtles have been bred at this facility since 1987 (Norman Soule, personal communication). The eggs were incubated and hatched at the hatchery and held for 2 weeks before being transported to the NYSDEC Aquatic Toxicant Research Unit (ATRU) and held for an additional 2 weeks prior to testing. The hatchling turtles were held in tanks containing 4 cm of sand substrate in which the turtles could burrow and with a slow flow of NYSDEC Rome Fish Hatchery spring water (Oneida County, NY). Water used in the tests (pH = 8.10, hardness = 132 mg/L $CaCO_3$, alkalinity = 117 mg/L $CaCO_3$, conductivity = 299 µmho/L (Paul 1997)) was kept at a depth of 4 cm above the substrate. The age of the turtle hatchlings at the beginning of the tests was 4 to 6 weeks. Carapace length was 45 mm (range 42 to 47 mm) and weight was 9.4 g (range 7 to 11 g). Turtles were allowed to feed *ad libitum* on black worms (*Lumbriculus variegatus*) and amphipods (*Gammarus* sp.) during this pre herbicide exposure holding time. The turtles remained in excellent health during the holding time, and only one turtle died (1%) shortly after transport to the ATRU.

Static non-renewal toxicity tests (Weber 1993) were conducted in order to approximate natural conditions following the herbicide treatment of a lake. In New York State, lakes treated with contact herbicides such as diquat and endothall are usually treated once a season and do not typically receive a continuous addition of herbicide over a number of days. The spot treatments performed with these herbicides typically result in short-term exposures within the treatment site due to dispersion and dilution and microbial degradation (endothall) or adsorption to particulates (diquat). The toxicity tests were conducted using 20-L glass containers with 16 L of test solution. No sediment was placed into test containers during the herbicide testing. Test chambers were held in a thermostatically controlled water bath, and the temperature was monitored continuously (19.0 ± 1.0°C). All concentrations were tested in duplicate with five turtles exposed per test container (i.e. a total of 10 turtles exposed to each herbicide concentration). Test containers were not aerated, and test animals were not fed during exposure to the herbicides. The test concentrations for diquat were 0, 1.0, 5.0, and 25.0 mg/L. The test concentrations used for potassium endothall were 0, 5.0, 25.0, and 125 mg/L. These concentrations represent a control concentration, plus exposure to the maximum application rate, 5 times the maximum application rate, and 25 times the maximum application rate for each herbicide. Nominal concentrations were used for all statistical calculations. NOECs (No-Observed-Effect Concentrations) were calculated following procedures described in Weber (1993).

Following exposure to the herbicides for 96 hrs, all turtles were measured, weighed, and returned to the flow-through tanks for 6 weeks post exposure monitoring. Turtles were fed as before during the post exposure monitoring time. Turtles that were visible in the sand substrate were checked for mortality or obvious signs of distress when fed (Monday, Wednesday, and Friday each week). All turtles were checked on a weekly basis for mortality and signs of distress.

RESULTS AND DISCUSSION

Neither diquat nor potassium endothall produced observable toxic affects on any of the turtles, and none of the test turtles died during any part of the exposure/post-exposure portions of the test. The turtles appeared in good health during the entire exposure and post-exposure period, and all of the turtles responded similarly to the control test animals. All of the turtles, regardless of the herbicide or concentration to which they were exposed, showed similar interest in food and fed as they had prior to testing. We observed no difference in the behavior of the test turtles with regard to burrowing in the sand substrate following herbicide exposure, with a high percentage of turtles burrowing to cover their carapaces. Based upon these studies, the NOEC for acute exposure to these herbicides is 25 mg/L and 125 mg/L for diquat and potassium endothall, respectively.

Softshell turtles are far less sensitive to diquat than many other aquatic species that have been tested. The freshwater amphipod *Hyalella azteca* is one of the most sensitive invertebrates to diquat, with a 96-h LC50 of 0.048 mg/L (Wilson and Bond 1968). Researchers have reported 96-h LC50s of 0.75 to 300 mg/L to a variety of fish species (Hiltibran 1967, Hughes 1975, Simonin and Skea 1977, Johnson and Finley 1980, Williams et al. 1984, Paul et al. 1994). Paul et al. (1994) reported 96-h LOECs (Lowest-Observed-Effect Concentrations) of 0.93, 3.4, and 3.6 mg/L to walleye (*Sander vitreus*), smallmouth bass (*Micropterus dolomieu*), and largemouth bass (*Micropterus salmoides*) respectively. Exposure to concentrations of diquat of 0.75 mg/L resulted in reduced size and pigmentation of stage 22 to 24 *Xenopus* embryos (Anderson and Prahlad 1976). Dial and Bauer-Dial (1987) found that exposure of leopard frog embryos (*Rana pipiens*) to diquat concentrations of 5 mg/L resulted in significant mortality. Hatchling softshell turtles are orders of magnitude less sensitive to diquat than these other aquatic animals.

The toxicity of endothall is very dependent on the particular formulation. The alkylamine salts of endothall (Hydrothol formulations) are more persistent and more toxic to aquatic organisms (Keller et al. 1988, Pennwalt Corp. 1980). Inorganic salts (potassium or sodium) of endothall (Aquathol) formulations are far less toxic to aquatic animals. *Daphnia magna* have a 48-h LC50 of 72 mg/L to potassium endothall (Office of Pesticide Programs 2004). The range of 96-h LC50s reported for fish are from 16 to 580 mg/L for potassium endothall (Walker 1963, Johnson and Finley 1980, Mayer and Ellersieck 1986, Paul et al. 1994). Paul et al. (1994) found 96-h LOECs of 11, 45, and 100 mg/L for walleye (*Sander vitreus*), smallmouth bass (*Micropterus dolomieu*), and largemouth bass (*Micropterus salmoides*) respectively. Amphibian toxicity data for the potassium endothall formulation is lacking. Softshell turtles are at least an order of magnitude less sensitive than aquatic invertebrates and many fish species.

Since none of the turtles showed any ill effect after being exposed to either diquat or potassium endothall, it is highly likely that an LC50 for softshell turtles is at least 10 times greater than our highest test concentrations. This suggests that the LC50s for diquat and potassium endothall are at least 250 times the maximum application rates. It is unlikely that either of these aquatic herbicides pose a risk of toxic effect to softshell turtles. However, the alteration of the aquatic plant habitat caused by herbicide use might affect hatchling aquatic turtle behavior and subsequent survival from predation. Birds, especially gulls and herons as well as large predatory fish, such as bass, might find a newly hatched turtle a desirable prey item (Smith 1985). These pressures may also force turtles to move to new locations that provide additional hiding places.

Because of their unique anatomy and physiology, softshell turtles are likely to be among the most sensitive of aquatic turtles to pesticides which are present in the water column. Their skin is highly vascularized, allowing underwater respiration (Moll and Moll 2004). They also engage in buccopharyngeal respiration, flushing water in and out of their mouths. Gage and Gage (1886) report that the mucous membrane of the pharynx is filled with filamentous processes that resemble the gills in the mudpuppy (*Necturus maculosus*). They determined the average rate of water pumping as 16 times per minute. In addition, a softshell turtle can flush water into and out of its cloaca for respiration (Dunson 1960). This increases the potential for materials dissolved in the water to enter into a turtle. Due in part to their soft, leathery shells, softshell turtles have skin which is three to four times more permeable to water than other hard-shelled turtles. The skin of these turtles is so permeable that if they are held out of water for 2 to 3 days, a softshell turtle may die from dehydration (Ernst et al. 1994). So softshell turtles provide a test species of turtle that by its anatomy and physiology increases the probability of uptake of a pesticide or other contaminant present in water. This would tend to make the species more sensitive to herbicides that had been applied to a pond or lake. Our study however documents that softshell turtles were not sensitive to either diquat or endothall.

ACKNOWLEDGMENTS

We wish to thank Norm Soule of the Cold Spring Harbor Fish Hatchery and Aquarium for the hatchling turtles used in our study, David Gapp of Hamilton College for information regarding the care of the turtles, and Jerry Czech who first suggested there might be a problem.

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