Acute Toxicity of Triclopyr Herbicide to the Gastropoda Snails European Ambersnail, *Succinea putris* and Tadpole Physa, *Physella gyrina*.

Report Prepared For:

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November 19, 2009

INTRODUCTION:

The Town of Cazenovia, New York treated the north end of Cazenovia Lake during the summer of 2009 with the aquatic formulation of the herbicide triclopyr manufactured by SePRO Corporation, Carmel, IN. Renovate® OTF granular (Active Ingredient: triclopyr: 3,5,6-trichloro-2-pyridinyloxyacetic acid, triethylamine salt, 14.0%) was used in 2009 to treat 234 acres at the north end of Cazenovia Lake, and the Town has proposed to treat 173.5 acres at the south end of the Lake in 2010 (NYSDEC 2009). The goal of these treatments is primarily to eradicate the invasive aquatic plant Eurasian watermilfoil (*Myriophyllum spicatum*). The outlet from Cazenovia Lake is at the south end, so low concentrations of the active ingredient in Renovate® OTF will flow out of the Lake and ultimately down Chittenango Creek.

About 5.3 miles downstream from the Cazenovia Lake outlet on Chittenango Creek is Chittenango Falls, the home of the Chittenango Ovate Amber Snail (*Novisuccinea chittenangoensis*). The Chittenango Ovate is listed as endangered in NYS and threatened by the Federal Government.

There are no Gastropoda (snail) toxicity data in the literature to evaluate the potential threat to the Chittenango Ovate Amber Snail from the proposed herbicide use in Cazenovia Lake. The purpose of this project was to provide the necessary toxicity data to evaluate the potential threat of the proposed 2010 triclopyr herbicide treatment to the endangered Chittenango Ovate Amber Snail. Two species of Gastropoda will be tested to evaluate the potential for the proposed triclopyr herbicide treatment to harm the endangered Chittenango Ovate Amber Snail. *Physella gyrina,* Tadpole Physa, is a small aquatic pulmonate (air breathing) snail that lives primarily in streams and ponds (Harman and Berg 1971; Turgeon et al 1988), and the terrestrial snail *Succinea putris,* European Ambersnail. S. *putris* was first found at Chittenango Falls in 1984 (NYSDEC 2009a).

There may still be some scientific debate whether or not *S. putris* was somehow introduced at Chittenango Falls, or if it might be a variant of the Chittenango Amber Ovate Snail (personal communication, Dr. Steven Campbell).

MATERIALS AND METHODS:

P. gyrina were collected from Spring Brook, Caledonia, NY on May 26, 2009. The author's tentative species identification was confirmed by the New York State Department of Environmental Conservation Stream Biomonitoring Unit. These feral snails were used to start a laboratory culture of *P. gyrina* for use in toxicity tests with triclopyr. The snails were cultured in a 38 liter glass aquarium with 10 L of standard, synthetic moderately-hard water (hereafter referred to as reconstituted water) (USEPA 2002) under static renewal conditions. The *P. gyrina* were fed Tetramin® Tropical Flake Food and cultured *Selenastrum capricornutum* green algae *ad libitum*. The culture water was changed weekly.

Succinea putris, European Ambersnails, were collected July 2 and August 27, 2009 from water celery beds, *Oenanthe japonica,* located immediately downstream from Chittenango Falls in Chittenango Falls State Park. During both collections, identifications were confirmed by Dr. Seven Campbell, a State University of New York at Syracuse Environmental Science and Forestry School post-doc with a grant to study the endangered Chittenango Ovate Amber Snail at Chittenango Falls. Dr. Campbell was present at both collection times and confirmed the *S. putris* identification. Approximately 240 *S. putris* were collected on July 2 and about 220 on August 27, 2009.

The snails from both collection dates were held in a modified 12-gallon plastic storage tote with a snap-tight lid. A 8 x 46 cm rectangle hole was cut in the center of the lid, and a 0.6 cm thick piece of clear acrylic plastic was glued and screwed in place to cover the hole. A 46 cm long cool-white 15-watt under-the-counter fluorescent lamp shown through the clear acrylic window for light (time controlled 16h light, 8h dark; ~ 80 ft-c intensity). Four 8 cm diameter holes were cut in each corners of the lid and covered with 1.5 mm mesh nylon netting for ventilation. The bottom was covered with washed quartz gravel from 2-6 cm in diameter, and sprigs of water celery, *O. japonica*, loosely covered the gravel to a depth of about 6 cm. The *S. putris* were misted *ad libitum* with reconstituted water from a plastic spray bottle to keep holding container, vegetation, rocks and snails moist, which simulated conditions where they were collected at Chittenango Falls. Fresh water celery was added about every two weeks.

The *S. putris* collected July 2, 2009 lived about 4 weeks. After two weeks of holding, the author could not find a local source of water celery, so water cress, *Nasturtium officinale,* was substituted. The European Ambersnails appeared to avoid this new food and cover, and all died within two weeks. The S. *putris* collected August 27, 2009 lived until they were euthanized about a week after completion of the definitive triclopyr toxicity test on September 13, 2009. They were provided fresh water celery weekly from Spring Brook, Mumford, NY. *S. putris* appear to prefer and survive better on water celery than they do on water cress.

The triclopyr used in these tests was 100% triclopyr acid technical received from Dow Agrosciences LLC on August 14, 2009.

Preliminary range finding tests were performed a few days before the definitive acute toxicity tests with both *P. gyrina* and *S. putris*. The range finding tests were similar to the definitive tests described here and outlined in Tables 1 and 2. The difference was the use of fewer test animals (5/concentration), no replication, and wider spaced concentrations. The objective of the range finding tests was to define the triclopyr concentration range necessary for the definitive test to ensure data that would allow calculation of 24-, 48-, 72- and 96-hour LC50 and/or EC50 statistical endpoints.

The definitive test conditions for *P. gyrina* and *S. putris* acute toxicity tests are summarized in Table 1 and 2, respectively. The *P. gyrina* toxicity test was a traditional aqueous renewal acute toxicity test with lethality/morbidity endpoints after 24, 48, 72 and 96-hours of exposure to the toxicant (USEPA 1975; USEPA 2002). Test solutions received 80% renewal every 24-hours to minimize effects from potential photo-degradation of triclopyr toxicant. The *S. putris* endpoints were the same as *P. gyrina*, but the exposure scenario closely mimicked the exposure conditions for *S. putris* and endangered *N. chittenangoensis* snails at Chittenango Falls. These snails live in the mist of the falls, so in the *S. putris* definitive acute toxicity test the snails were misted with various concentrations of triclopyr in reconstituted water (Table 2).

Dissolved oxygen and pH were analyzed in the test chambers just before and after renewal of test solutions in the *P. gyrina* definitive test.

RESULTS

The *P. gyrina* definitive acute toxicity test was performed on September 15 – 19, 2009, and the *S. putris* definitive acute toxicity mist test was performed on September 8 – 12, 2009.

Wet weight biomass loading in the *P. gyrina* test averaged 0.50 g/L (range 0.40 – 0.59 g/L), including the shell (at least 50% of total wet weight). ASTM (2002) recommendations that biomass loading in static and renewal-static tests at 22ºC be ≤0.4 g/L wet tissue weight, so the loading factor in this test was well within recommended levels. The biomass loading in the *S. putris* was somewhat higher at 1.46 grams per replicate (range 1.20 – 1.71 grams/replicate), including shell. There is no guidance for loading factors in a mist test from either ASTM or USEPA. The conditions in this mist test were meant to mimic conditions of triclopyr exposure at Chittenango Falls for both *S. putris* and *N. chittenangoensis*. Therefore, the loading factor in these tests was not a concern.

The dissolved oxygen concentrations in the *P. gyrina* definitive toxicity test averaged 7.5 mg/L, with a range of 7.1 – 8.0 mg/L. The average pH was 7.2, with a range of 7.1 – 7.4. Both dissolved oxygen and pH were in the range that would be expected. The mean

temperature was 21.7ºC, and the range was 21.7-22.1ºC. Neither dissolved oxygen nor pH readings were appropriate monitoring parameters for the *S. putris* mist test. The mean temperature was 22.2ºC, and the range was 21.6-22.8ºC.

The mortality/morbidity data for the *P. gyrina* and *S. putris* definitive toxicity tests are summarized in Tables 3 and 4, respectively. The highest test concentration was 400 mg/L triclopyr, which is very close to solubility (408 mg/L) of the 100% technical grade material used in these tests.

CETIS Software Package (CETIS 2006) was used for statistical analysis of the *P. gyrina* data. Trimmed Spearman-Karber was the statistical method used for the *P. gyrina* data. The LC50s after 24, 48, 72 and 96 hours of exposure were all the same. The LC50 was 293 mg/L, the 95% LCL (Lower Confidence Limit) was 284 mg/L, and the 95% UCL (Upper Confidence Limit) was 302 mg/L. No morbidity effects were observed with *P. gyrina*.

No statistical analysis of the *S. putris* mist test data was possible, as there was no mortality at the concentrations tested. The LC/EC50 was >400 mg/L, the approximate solubility of technical grade triclopyr in water.

DISCUSSION

The maximum label application rate for the proposed triclopyr formulation Renovate® OTF granular to control Eurasian watermilfoil is 2.5 mg/L active ingredient (triclopyr acid equivalent). The triclopyr herbicide is proposed to be applied to Cazenovia Lake in the 1-2 mg/L range. Dilution, attenuation, and decomposition is expected to reduce the concentration actually observed at Chittenango Falls to the 0.2 mg/L range or less (Cazenovia 2009). The estimated triclopyr concentration at Chittenango Falls is expected to be 3 orders of magnitude less than the no-effect concentration with *S. putris* and the LC50 concentration for *P. gyrina* was nearly 1,000 times higher than the expected concentration at Chittenango Falls. Therefore, there appears to be a wide margin of safety to protect the endangered Chittenango Ovate Amber Snail from any adverse effects from the proposed triclopyr herbicide treatment in Cazenovia Lake.

These toxicity results are similar to what would be expected for a plant growth regulator like triclopyr, and what has been reported in the literature. The acute LC50s reported for freshwater fish range from 44 mg/L for fathead minnow (slightly toxic) to 891 mg/L for bluegill (practically non-toxic). The reported acute toxicity for the water flea, *Daphnia magna*, was 1,496 mg/L. The only reported acute toxicity result for a mollusk species was the marine Eastern oyster, *Crassostrea virginica*, which had an EC50 of 58 mg/L, based on shell deposition (ENSR 2007).

Table 1. Summary of *P. gyrina* definitive acute toxicity test procedure.

1. Test type:	Static-renewal
2. Test duration	96-h; check mortality/morbidity at 24-, 48-, 72-, and 96-h
3. Temperature	22±1°C
4. Light quality	Cool-white fluorescent
5. Light intensity	90 ft-c
6. Photoperiod	16-h light, 8-h dark
7. Test chamber	200 x 80 mm glass culture dishes with acrylic plastic covers
8. Test solution vol.	1000 ml
9. Renew test soln's	80% renewal at 24-, 48- and 72-hours with fresh solutions
10. Test organism source	Lab culture; identification verified
11. # organisms/chamber	10
12, # replicates/conc.	2
13. # organisms/conc.	20
14. Feeding	None during test
15. Chamber cleaning	None during test
16. Test soln. aeration	None
17. Dilution water	Moderately-hard EPA synthetic water (pH 7.4-7.8; hardness 80-100; alkalinity 57-64)
18. Test concentrations	5 + control, depending on results of range finding tests; results based on nominal concentrations (not measured concentrations)
19. Dilution series	0.8 between dilution series of 400, 320, 256, 205, 164 & 0 mg/L
20. Endpoint	LC50 and/or EC50 as appropriate
21. Test acceptability	≥90% survival in controls

Table 2. Summary of *S. putris* definitive acute toxicity test procedure.

1. Test type:	Mist test to simulate exposure to triclopyr at Chittenango Falls
2. Test duration	96-h; check mortality/morbidity at 24-, 48-h, 72- and 96-h
3. Temperature	22±1°C
4. Light quality	Cool-white fluorescent
5. Light intensity	90 ft-c
6. Photoperiod	16-h light, 8-h dark
7. Test chamber	200 x 80 mm glass culture dishes with acrylic plastic covers
8. Test design	600 grams ¼" pea washed pea gravel covering bottom of beaker; sprig of water celery for food/habitat
9. Test solution delivery	Mist each container every 2-hours during daylight with different concentrations of triclopyr to simulate potential exposure at Chittenango Falls; remove excess solution that might collect in pea gravel daily with needle and syringe
10. Test organism source	Chittenango Falls water celery beds where author was able to collect 240 specimens in 20 minutes on July 2, 2009. Chittenango Ovate Snails have never been observed at that spot (per Steven Campbell, ESF).
11. # organisms/chamber	10
12, # replicates/conc.	2
13. # organisms/conc.	20
14. Feeding	None during test, except water celery
15. Chamber cleaning	None during test, except remove excess mist water
16. Test soln. aeration	N/A
17. Dilution water	Moderately-hard EPA synthetic water for mist preparation (pH 7.4-7.8; hardness 80-100; alkalinity 57-64)
18. Test concentrations	5 + control, depending on results of range finding tests; results based on nominal concentrations (not measured concentrations)
19. Dilution series	0.5 dilution series of 400, 200, 100, 50, 25 mg/L and 0 (control). Solubility of technical triclopyr is ~ 400 mg/L.
20. Endpoint	LC50 and/or EC50 as appropriate
21. Test acceptability	≥90% survival in controls

Table 3. Summary of mortality data from *P. gyrina* definitive acute toxicity test.*

Conc-mg/L	Rep	# Exposed	24h Survival	48h Survival	72h Survival	96h Survival
0	1	10	10	10	10	10
0	2	10	10	10	10	10
164	1	10	10	10	10	10
164	2	10	10	10	10	10
205	1	10	10	10	10	10
205	2	10	10	10	10	10
256	1	10	10	10	10	10
256	2	10	10	10	10	10
320	1	10	1	1	1	1
320	2	10	1	1	1	1
400	1	10	0	0	0	0
400	2	10	0	0	0	0

***** There were no observations of morbidity (i.e., unable to right animal on foot). All surviving *P. gyrina* acted normal. Individuals were either alive and normal, or dead on bottom of test container with no response of foot when stimulated.

Table 4. Summary of mortality data from *S. putris* definitive acute toxicity test.*

Conc-mg/L	Rep	# Exposed	24h Survival	48h Survival	72h Survival	96h Survival
0	1	10	10	10	10	10
0	2	10	10	10	10	10
25	1	10	10	10	10	10
25	2	10	10	10	10	10
50	1	10	10	10	10	10
50	2	10	10	10	10	10
100	1	10	10	10	10	10
100	2	10	10	10	10	10
200	1	10	10	10	10	10
200	2	10	10	10	10	10
400	1	10	10	10	10	10
400	2	10	10	10	10	10

* No mortality or morbidity observed in this test.

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