A Review of the Toxicity and Environmental Fate of Triclopyr

Submitted to the Massachusetts Pesticide Board Subcommittee

By Steven E. Antunes-Kenyon and Gerard Kennedy
Massachusetts Department of Agricultural Resources
November 12, 2004

EXECUTIVE SUMMARY

The purpose of this report is to review the environmental fate and toxicity of the pesticide active ingredient, Triclopyr, when formulated for aquatic weed control. While triclopyr has been registered for many years in Massachusetts for terrestrial uses, it has only recently been registered at the federal level for weed management in lakes and ponds. In 2004, SePRO submitted the triclopyr product "Renovate 3 Aquatic Herbicide" (EPA # 62719-37-67690), for registration in Massachusetts. Because the Renovate 3 product represents a significant new use pattern for triclopyr, the Massachusetts Pesticide Board Subcommittee directed the Pesticide Bureau to undertake a special review.

Renovate 3 Aquatic Herbicide is formulated as a Triclopyr Triethylamine (TEA) salt. As a general use pesticide product, it is labeled for control of floating, immersed, or submersed aquatic plants in and around aquatic sites such as ponds, lakes, reservoirs, non-irrigation canals, ditches, marshes and wetlands. Triclopyr is a systemic herbicide with selective control of woody and broadleaf species. In aquatic ecosystems, this differential response gives triclopyr the ability to remove milfoil and allow non-invasive native monocots and tolerant dicots to proliferate. The maximum label application rate results in a concentration of 2.5 ppm in the water body.

There are no restrictions on the use of water in the treatment area for recreational purposes, including swimming, and fishing; or on the use of treated water by livestock from treatment areas. There are, however, label restrictions on the use of treated water for irrigation and on applications where there are potable water intakes. For potable water intakes, the label outlines protective buffers. For floating and emerged weeds, setback distances range from 0 to 1300 feet depending upon the amount of area treated. For submerged weeds, a chart is used to determine the minimum setback distances.

Environmental Fate:
Triclopyr triethylamine salt (TEA) is highly soluble in water and dissociates within one minute to the weak acid, triclopyr. Aquatic photolysis and microbial breakdown are significant degradation pathways for triclopyr. Dissipation half lives of triclopyr in water range from 0.5 days to 7.5 days. In sediment, triclopyr dissipation rates ranged from 2.8 to 5.8 days in field studies. Triclopyr is, however, persistent under anaerobic aquatic conditions. It is highly water soluble and is not expected to bind with organic materials.

Toxicity:
This review includes an overview of the available triclopyr ecotoxicity database. Although much of the data from laboratory ecotoxicity testing limits the complexity of stress parameters, except perhaps for isolating the toxicant, this review does include data from available and pertinent field study investigations.

Carcinogenicity : A DEP/DAR review noted positive results in oncogenicity studies in female rats at high dose levels. However EPA scientists felt that the animal carcinogenicity evidence was marginal (not entirely negative, but yet not convincing) resulting in triclopyr being classified as a Group D chemical (not classifiable as to human carcinogenicity).

Acute Toxicity: The results of acute toxicity studies conducted with triclopyr TEA formulation indicate that the material is highly corrosive to the eyes and slightly toxic to relatively non-toxic via other routes of exposure.

Dietary Exposure from Use of Renovate 3: No significant contributions to dietary exposure are expected from the use of Renovate 3.

Exposure from Drinking Water and Recreational Uses of Treated Water: Given that triclopyr residues in water degrade rapidly via photolysis, the risks from exposure to triclopyr via drinking water or recreational uses should be negligible.

Amphibian Toxicity: Garlon 4 (triclopyr BEE) shows significantly greater toxicity to *Xenopus Laevis* embryos as compared to Garlon 3 (triclopyr TEA) (Perkins, 2000). Observations and data indicated a trend of increased Release® (triclopyr BEE) toxicity to amphibians under decreased pH. The combination of Release® and pH was not deemed to be teratogenic.

Freshwater Invertebrates Toxicity: Triclopyr acid is practically non-toxic to freshwater invertebrates. Based on the waterflea (*Daphnia magna*) life-cycle toxicity study using triclopyr TEA formulation, the calculated 48-hr LC_{50} value based on nominal concentrations, was 1,170 ppm and the 21-day chronic toxicity LC_{50} value, based on analyzed concentrations, was 1,140 ppm. Thus there are acceptable margins of safety assuming an EEC$\leq$2.5 ppm.

Estuarine and Marine Animal Toxicity: The results indicate that triclopyr TEA is slightly toxic to practically non-toxic to estuarine/marine invertebrates on an acute basis and practically non-toxic to estuarine/marine fish on an acute basis. However, the Renovate 3 product labeling does prohibit application to salt water or estuaries.

Fish Toxicity Data: Both triclopyr acid and triclopyr TEA are practically non-toxic to freshwater fish on an acute basis. Triclopyr TEA has fish 96-hr LC_{5O} values of 552 and 891 ppm for rainbow trout and bluegills respectively. The corresponding values for triclopyr acid are 117 and 148 ppm for rainbow trout and bluegill respectively. Thus, there are acceptable margins of safety assuming an EEC$\leq$2.5 ppm.

Mammals: Studies reviewed show that triclopyr acid is practically non-toxic to small mammals on an acute oral basis.

Birds: Triclopyr presents low acute and subchronic toxicity to the bird species tested. According to the 1998 EPA RED, reproduction of birds may be affected at levels greater than 100 ppm of triclopyr TEA (p.38). Waterfowl are likely to be the most highly exposed bird species, given that they swim, drink and feed on lakes and ponds proposed for treatment with Renovate 3. Given the maximum expected environmental concentrations of 2.5 ppm, the rapid degradation in treated water, and the lack of bioaccumulation, there are negligible risks to avian species, including those whose diet might consist primarily of aquatic vegetation treated with triclopyr.

Plant Toxicity: Similar to other currently registered and used aquatic herbicides (see *Appendix D: Alternative Control Materials*), triclopyr is designed to be toxic to plants, especially woody

and broadleaf species. By default, it therefore presents some risk to threatened or endangered aquatic plants. Results from reviewed studies, indicate that triclopyr is only slightly toxic to the native aquatic macrophyte, duckweed (*Lemna gibba)*. The available data indicate that at the maximum EEC, native duckweed had an average inhibition of ~ 23%; however, many species of cyanobacteria and algae actually showed stimulation to growth as compared to controls.

Field dissipation studies indicate that triclopyr accumulation in sediments, shellfish and fish is negligible.

Aquatic aerobic degradation studies and field dissipation studies have shown the formation of the primary metabolite: 3,5,6-trichloropyridinol (TCP). TCP is also a metabolite of the closely related (analog) insecticide chlorpyrifos. Whereas triclopyr acid is practically non-toxic to fish, TCP is moderately toxic to some aquatic species. Lab studies using technical grade TCP provide a Rainbow trout 96-hr LC_{50} of 1.5 ppm (see Table 20: Acute Toxicity of TCP to Freshwater Fish). Nevertheless, based on the triclopyr EEC from labeled uses of Renovate 3, the environmental fate properties of triclopyr, and data from a number of additional fish toxicity studies as outlined below, TCP is not expected to present an unreasonable risk to aquatic species:

- Maximum label application rates of triclopyr cannot result in TCP water concentrations greater than the parent triclopyr acid (EEC of 2.5 ppm);
- TCP field dissipation studies show half lives from 4.2 days to 10 days;
- TCP rapidly degrades into nonhalagenated, low molecular weight organic acids;
- TCP has a water solubility of 49,100 ppm indicating relatively high hydrophilicity, low potential to partition to lipid material, and rapid elimination from aquatic organisms;
- There were no biologically significant indications of acute physiological stress in juvenile Coho Salmon (*Salmo gairdneri*) exposed for 4-hours to Garlon 3A (a.i. triclopyr TEA) at nomimal concentrations up to 400 ppm (80% of the juvenile rainbow trout 96-hr LC_{50}); and
- The Fathead minnow (*Pimephales promelas*) - fish early-life stage toxicity test with triclopyr TEA resulted in an estimated Maximum Acceptable Toxicant Concentration (MATC) of 130 ppm.

In summary, strict adherence to Renovate 3 labeling, will result in minimal acute and negligible chronic risks to most fish, waterfowl, amphibians and aquatic invertebrates from triclopyr TEA and its metabolites. However, use of Renovate 3 in wetlands may result in significant risks to threatened and endangered aquatic plant species. Strict adherence to product labeling with oversight via the State's wetlands protection laws, permit requirements for nuisance aquatic vegetation, and endangered species program requirements, are adequate to manage these risks in sensitive areas.

The Pesticide Bureau recommends that the Pesticide Board Sub-Committee register Renovate 3 for aquatic weed control in Massachusetts.

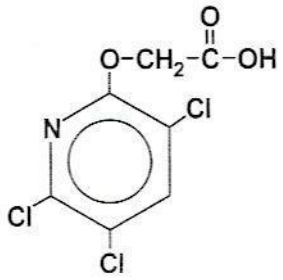
1. Product Information

Renovate 3 Aquatic Herbicide (EPA Reg. No. 62719-37-67690), is distributed by SePRO (distributor number 67690) of Carmel, Indiana. This formulation of triclopyr is sold to SePRO by Dow AgroSciences. Dow markets this triclopyr formulation for terrestrial uses under the name "Garlon 3A" (EPA Reg. No. 62719-37).

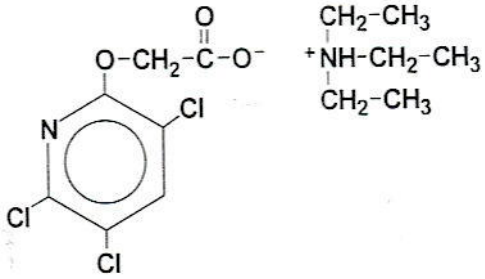
Renovate is formulated as the triethylamine (TEA) salt of triclopyr. The liquid formulation consists of 44.4% triclopyr: 3,5,6-trichloro-2-pyridinyloxyaceticacid, triethylamine salt. The acid equivalent[1] is, triclopyr 31.8% (or 3lb/gal). "Inert" ingredients include ethanol; triethylamine and EDTA.

Triclopyr is a pyridine carboxylic acid differing from 2,4,5-T only by the presence of a nitrogen in the ring structure. Like 2,4,5-T, triclopyr is a synthetic plant growth hormone, or auxin, that interferes with plant metabolism. The chemical has been registered since the mid-1970's in the U.S. for control of broadleaf weeds and wood plants on rights-of-way, rangeland, industrial sites and other non-crop areas.

While the parent molecule of triclopyr is an acid, it is formulated in Renovate 3 as an amine/salt derivative. Generally acid molecules are formulated as salts, esters or amines to enhance their absorption by the plant leaf or increase their solubility. The Renovate formulation is readily miscible in water[2]. The parent acid is the herbicidally active portion of the formulation, binding to the herbicide target site within the plant leading to plant death. The salt or ester portion of the formulated product plays no role in binding to the herbicide target site.



Triclopyr acid



Renovate 3 Formulation:
Triclopyr triethylamine salt (TEA)

Application Sites

In addition to lakes, ponds, reservoirs, and non-irrigation canals or ditches, Renovate 3 is labeled for use in wetlands including flood plains; deltas; marshes; swamps; bogs; transitional areas between upland and lowland sites, including wetlands that occur within forests; wildlife habitat

[1] **Acid Equivalent**:. Triclopyr acid is formulated as a salt in Renovate 3. The salt represents the product "active ingredient" while the acid equivalent represents the original acid portion of the molecule which is the herbicidally active portion. Acid Equivalent is equal to molecular weight of the acid, minus 1, divided by the molecular weight of the triclopyr TEA or triclopyr BEE, multiplied by 100.

[2] For comparison, note the water solubility for the various chemical forms of triclopyr: parent acid = 430 mg/L; Butoxy Ethyl Ester (Garlon 4) = 23 mg/L; and TEA 2,100,000 mg/L at 25 ^{o}C (Vencill, 2002).

restoration and management areas and similar sites; and areas adjacent to or surrounding domestic water supply reservoirs, lakes and ponds.

Hazards and Warnings

Relative to potential human hazards, Renovate 3 bears the signal word DANGER due to its potential to cause irreversible eye damage. Protective eyewear as well as long-sleeved shirt and long pants, shoes plus socks, and chemical resistant gloves are required Personal Protective Equipment.

The Environmental Hazards section of the labeling discusses the potential for depleted oxygen (low dissolved oxygen) as a result of decomposition of treated weeds. Under some conditions, such effects may lead to fish kills. Related to this concern, the labeling states that applicators should not treat more than one-third to one-half of the water area in a single operation and wait at least 10 to 14 days between treatments. It should also be noted that due to the relatively low toxicity of triclopyr TEA to wildlife, the Environmental Hazards section of this labeling does not include precautionary label statements relative to toxicity to aquatic invertebrates, fish, or waterfowl.

Mode of Action

Triclopyr, along with other herbicides such as clopyralid, fluroxypyr, and picloram, is classified as a pyridine carboxylic acid. Although not completely understood, the primary action of these compounds is thought to be like that of the naturally occurring auxin, Indole Acetic Acid (IAA) The action appears to involve cell plasticity and nucleic acid metabolism. The symptoms typical of auxin-type herbicides include epinastic[3] bending and twisting of stems and petioles, stem swelling (particularly at nodes) and elongation, and leaf cupping and curling (Vencill, 2002).

Triclopyr's auxin-type herbicidal activity generally controls woody and broadleaf species while most grasses and other monocots are tolerant (WSSA, 2002). In aquatic ecosystems this differential response gives triclopyr the ability to remove milfoil and allow non-invasive native monocots and tolerant dicots to proliferate and provide wildlife habitat, sediment stabilization, and nutrient cycling (Sprecher, 1995).

Triclopyr has potential for management of invasive weeds such as Eurasian watermilfoil (*Myriophyllum spicatum* L.) and other susceptible submerged weeds in lakes, ponds, reservoirs, and in non-irrigation canals or ditches that have little or no continuous outflow. It may also prove useful in control of purple loosestrife (*Lythrum salicaria* L.), because of its ability to control these dicot species selectively.

As a systemic herbicide, killing the entire plant including the roots, triclopyr will generally provide longer efficacy than contact aquatic herbicides, such as endothall, which leave roots alive to regrow.

[3] Epinastic refers to growing faster on one side of the leaf or stem than the other.

Aquatic Weeds Controlled by Renovate 3

According to the product labeling, the following aquatic weeds are controlled with Renovate:

Alligatorweed	American lotus
Milfoil species	Pennywort
Purple loosestrife	Waterlily
nuphar (spatterdock)	Aquatic sodaapple
Waterhyacinth	Pickerelweed
American frogbit	Waterprimose
Parrotfeather	Eurasian watermilfoil

Table 1: Aquatic Weeds Controlled by Renovate 3

Applications may be made directly to water bodies and/or plants actively growing in and around such water via backpack sprayer, boat, helicopter, spray boom, handgun or other suitable equipment. The use of mistblowers is not recommended nor may applications be made via chemigation.

Floating and Emerged Weed Control

Renovate 3 is labeled for control of floating and emerged weeds, such as waterhyacinth, alligatorweed, purple loosestrife, as well as other woody plants such as poison ivy, maples, and black gum in and around lakes, reservoirs or ponds. For use of Renovate 3 in these areas that also contain a functioning potable water intake for human consumption, the product labeling includes the following table to determine the minimum setback distances of the application from the functioning potable water intakes:

	Renovate 3 Application Rate, qt/acre			
Area Treated (acres)	**2qt/ acre**	**4qt/ acre**	**6qt/ acre**	**8qt/ acre**
	Setback Distance (ft)			
<4	0	200	400	500
>4-8	0	200	700	900
>8-16	0	200	700	1000
>16	0	200	900	1300

Table 2: Floating and Emerged Weeds Control -
Minimum Setback Distances from Functioning Potable Water Intakes

As per the table above there are no label required minimum setbacks for applications at the 2 qt./A treatment level. At this rate of application, the maximum nominal concentration of triclopyr ae immediately following the application to 1-acre of water that is 1-foot deep is ~0.55 mg/L (ppm).

Submerged Weed Control

Renovate 3 is labeled for control of submerged weeds, such as watermilfoil (*Myriophyllum spicatum*) in lakes, reservoirs or ponds, and in non-irrigation canals or ditches that have little or no continuous outflow. For control of susceptible submerged weeds in such sites, the application rates are provided in Table 3:

Water Depth (ft)	Concentration of Triclopyr Acid in Water (ppm acid equivalent)				
	0.75 ppm	1.0 ppm	1.5 ppm	2.0 ppm	2.5 ppm
	Gallons of Renovate 3 per Surface Acre at Specified Depth				
1	0.7	0.9	1.4	1.8	2.3
2	1.4	1.8	3.3	3.6	4.6
3	2.1	2.9	4.1	5.4	6.8
4	2.7	3.6	5.4	7.2	9.1
5	3.4	4.5	6.8	9.0	11.3
6	4.1	5.4	8.1	10.9	13.6
7	4.8	6.3	9.5	12.7	15.8
8	5.5	7.2	10.9	14.5	13.1
9	6.1	8.1	12.2	16.3	20.4
10	6.8	9.0	13.6	18.1	22.6
15	10.2	13.6	20.4	27.2	33.9
20	13.6	18.1	27.2	36.2	45.3

Table 3: Applications rates to Ponds, Lakes, Reservoirs, and Non-irrigation Canals, and Ditches

For control of submerged weeds in areas where there is a functioning potable water intake, the following chart and formulae must be used to determine the minimum setback distances of the application from the intake:

Concentration of Triclopyr Acid in Water(ppm ae)					
Area Treated (acres)	0.75 ppm	1.0ppm	1.5ppm	2.0 ppm	2.5 ppm
	Required Setback Distance (ft) from Potable Water Intake				
< 4	300	400	600	800	1000
>4-8	420	560	840	1120	1400
>8-16	600	800	1200	1600	2000
>16 - 32	780	1040	1560	2080	2600
>32 acres, calculate a setback using the formula for the appropriate rate	Setback (ft) = (800 * ln (acres) – 160) / 3.33	Setback (ft) = (800 * ln (acres) – 160) / 2.50	Setback (ft) = (800 * ln (acres) - 160) / 1.67	Setback (ft) = (800 * ln (acres) - 160) / 1.25	Setback (ft) = (800 * ln (acres) - 160)

Table 4: Submerged Weed Control and Minimum Setback Distances from Functioning Potable Water Intakes

The labeling states that existing potable water intakes, which are no longer in use, such as those replaced by potable water wells or connections to a municipal water system, are not considered to be functioning potable water intakes. Also exempt from the labeling setback restrictions are terrestrial applications made adjacent to potable water intakes.

To apply Renovate 3 around and within the distances noted above from a functioning potable water intake, the intake must be turned off until the triclopyr level in the intake water is determined to be 0.4 ppm or less by laboratory analysis or immunoassay[4].

The labeling does not bear any restrictions on livestock consumption of water from the treatment area or on use of water in the treatment area for recreational purposes, including swimming and fishing.

Restrictions

Treated water may not be used for irrigation for 120-days following application. Alternatively, treated water may be used for irrigation once the triclopyr level in the intake water is determined to be non-detectable by laboratory analysis (immunoassay). Since triclopyr does not affect grasses, there is no restriction on use of water from the treatment area to irrigate established grasses; however, applicators must not allow Renovate 3 to come into direct contact with grapes, tobacco, vegetable crops, flowers, or other desirable broadleaf plants, and must not permit spray mists containing it to drift into them. Significant guidance relative to drift mitigation is found in the labeling for the different application methods approved.

The following directions and restrictions are also included in the labeling:

- Do not apply to salt water bays or estuaries.
- Do not apply directly to un-impounded rivers or streams.
- Do not apply on ditches or canals used to transport irrigation water. It is permissible to treat non-irrigation ditch banks.
- Do not apply where runoff water may flow onto agricultural land.
- When making applications to control unwanted plants on banks or shorelines of moving water sites, minimize overspray to open water.
- Applications must begin along the shore and proceed outwards in bands to allow fish to move into untreated areas.

State Permit Requirements

The labeling states that applicators must consult with appropriate state or local water authorities before applying to public waters to determine if permits are needed. Authority has been granted to the Massachusetts Department of Environmental Protection (DEP) by MGL ch. 111, s. 5E to issue licenses to apply chemicals for the control of nuisance aquatic vegetation. According to this statute, no person shall, for the purpose of controlling algae, weeds and other aquatic nuisances therein, apply chemicals to a lake, pond, stream, or other body of water within the territorial limits of the Commonwealth without first obtaining a license from DEP. For additional state requirements see the Ecotoxicity section of this review relative to threatened and endangered species.

In Massachusetts, a permit is not required for state or federal agencies while in the conduct of their official duties. No permit is required for privately owned (single owner) ponds from which there is no flowing outlet.

[4] SePro Corporation sells a Renovate Test, Enzyme-Linked Immumoassay (ELISA Test) for the determination of the active ingredient concentration in the water. Samples may also be collected and sent to SePro Corporation for analysis.

2. Environmental Fate

Triclopyr triethylamine salt (TEA) is highly soluble in water and dissociates within one minute to the weak acid, triclopyr and triethanolamine. Triethanolamine is stable under aquatic conditions undergoing microbial degradation to carbon dioxide, (half life: 14-18 days) (USEPA, 1998 (i)).

Triclopyr acid is stable to hydrolysis and anaerobic aquatic conditions. Laboratory tests show that aquatic photolysis is a significant degradation pathway for triclopyr. Field dissipation studies indicate that microbial mediated degradation is also important. Triclopyr degrades slowly under aerobic aquatic conditions to TCP. TCP has been shown in laboratory experiments to decompose rapidly upon exposure to UV radiation (half life 25 min) producing carbon dioxide and many degradation products (Feng, 1998).

The high water solubility of triclopyr acid (430 ppm) along with its partition coefficient values indicate that both triclopyr (Koc 27mg/L) and TCP (Koc 151 mg/L) are likely to be mobile in soil and not adsorb to organic materials or sediment. Terrestrial field dissipation studies confirm this with triclopyr detected at depths up to 45 cm. The environmental fate properties are summarized in Table Five:

Water Solubility	**Acid:** 430 ppm **TEA**: 2,100,000 ppm (WSSA, 2002) **TCP**: 49,100 ppm (Knuteson, 1999)	
Vapor Pressure	**Acid**: 1.60 x 10^{-7} kPa at 25^{o}C (1.26 x 10^{-6} mm Hg at 25^{o}C (WSSA, 2002)	
Partition Coefficients	**Acid**: Koc: 27 to 384mL/g average **Acid:** Kow: 0.204 (Knuteson, 1999)	
Hydrolysis	**Acid**: Stable	**TCP:** Stable
Aqueous Photolysis	**Acid:** 1.3 days	**TCP**: 2 hours (Knuteson, 1999)
Aquatic Aerobic Metabolism (half life)	**Acid:** 142 days (half life)	
Aquatic Anaerobic Metabolism (half life)	**Acid:** 1300 days (USEPA, 1998)	
Aquatic Field Dissipation (DT 50) (Water)	DT 50: 0.5 to3.5 days (Lake Seminole, GA) DT 50: 3.7 to 4.7 days (Lake Minnetonka,MN)	

Table 5 : Chemical and Environmental Fate Properties of Triclopyr and TCP

Laboratory Studies

Photolysis:
Laboratory studies showed that photolysis is a significant degradation pathway for triclopyr in aquatic environments. Photolysis studies were conducted in ph7 buffered water and river water respectively, under both artificial light and natural sunlight at 40 deg N latitude midsummer. Half lives averaged 0.5 days and 1.3 days for the buffered water and river water respectively. The difference, according to the study report author, is probably due to the presence of dissolved organic matter in river water.

Identified degradates in both river water and sterile water were 5-chloro-3,6-dihydroxy-2-pyridinoloxyacetic acid and oxamic acid. The principal degradation product in sterile water is 5-chloro-3,6-dihydroxy-2-pyridinoloxyacetic acid (up to 48% of applied). The photolysis of triclopyr in river water generated oxamic acid as the major photoproduct (16% of applied) along with several other low molecular weight carboxylic acids (Woodburn, 1993).

The suggestion that photolysis is the major degradation pathway for triclopyr has implications for degradation rates where light intensity is weak. Such conditions might be found on overcast days, or under turbid water conditions. In a laboratory exercise, triclopyr was observed to photodegrade in an aquatic environment with midday, midsummer half lives of approximately a couple of hours at the surface to 14 hours at 1 meter depth in the winter (Table 6).

Season and Depth	Triclopyr Half Life	Season and Depth	Triclopyr Half Life
Spring, surface	2.8 days	Fall, surface	4.6 days
Spring 1m	3.7	Fall, 1m	6.2
Summer, Surface	2.1	Winter, surface	10.6
Summer, 1m	2.8 days	Winter 1m	14.1 days

Table 6: Calculated midday, seasonal half lives of triclopyr at 400 N latitude (McCall and Gavitt, 1986).

However, according to reviews provided by Dow, field studies suggest that photolysis may play a more limited role in the breakdown of triclopyr. A study by Foster shows that most of the UV light needed to photolyze triclopyr is quenched within the first 10 to 25 cm of the water column (Foster, 1997). The field studies show rapid degradation in areas of heavy weed infestation, where light is poor. Because the quenching of light does not appear to significantly impact degradation in field studies, Houtman suggests that the primary mechanism for the removal of triclopyr from the aquatic environment is microbial degradation (Houtman, 1997).

TCP also degrades photolytically though many degradation products remain in the aqueous medium. Feng et al suggest that a consortium of microorganisms coupled with photolytic activity may be needed to achieve complete mineralisation of TCP and its degradates (Feng et al, 1998).

Aerobic Aquatic Metabolism: Under aerobic conditions triclopyr acid degraded slowly with a half life of 142 days. The only degradate observed was TCP at less than 5% of the applied.

Anaerobic Aquatic Metabolism: Transformation under anaerobic aquatic conditions is not a degradation pathway of any significance. From the EPA RED, triclopyr acid is persistent under anaerobic conditions decreasing to approximately 80% of the applied after a year. The registrant calculated half life is 1300 days.

Hydrolysis: Hydrolysis is not a significant breakdown pathway for triclopyr.

Mobility: The high solubility of triclopyr, 430 ppm, and the partition coefficient values indicate that both triclopyr (Koc 25 to 384mL/g) and TCP (Koc 14 to 86mL/g) are likely to be very mobile in soil and not adsorb to organic materials or sediment (EPA, 1996) (Hamaker, 1975). In

terrestrial field dissipation studies, low concentrations of triclopyr were found at soil depths of up to 45 cm, however triclopyr did not persist (EPA, 1998(i)).

Field Dissipation Studies

Summaries of field studies provided by Dow indicate that dissipation half lives of triclopyr in water range from 0.5 days to 7.5 days. For TCP, rates range from 4.2 days to 10 days. In sediment, triclopyr dissipation rates ranged from 2.8 to 5.8 days. For TCP, the rates were from 3.8 days to 13.3 days. The study summaries reviewed for this report were conducted in Lake Seminole, Georgia and Lake Minnetonka Minnesota. Additional studies reviewed were conducted in man made closed ponds in California, Texas and Missouri. A profile of the studies is provided in Table 7.

Lake Seminole, GA

A dissipation study was conducted in 10A plots in the summer at Lake Seminole, Georgia (31^{o}N Latitude; water temperatures of 31^{o}C[5]; average water depth is 4 feet). Both plots were heavily infested with submersed aquatic vegetation, including watermilfoil. Triclopyr, applied to achieve a nominal triclopyr water concentration of 2.5 mg/L, was found to dissipate with an average half life of 0.5 to 3.5 days at the surface. The average first order half life for triclopyr in water sampled from below the surface was 3.7 days. The metabolite, TCP, dissipated with a half life of less than a day. Complete aqueous dissipation of chemical (triclopyr and TCP) from the application areas took 42 days. The variability in dissipation half life rates was attributed to factors such as water movement, vegetative cover, and the type of vegetation. Low levels of triclopyr residues were detected in sediment after the initial treatment. However, neither triclopyr nor TCP was found to accumulate in sediment. Typical values of dissolved oxygen and pH observed were 8ppm and 8.4 respectively. (Woodburn, 1988)

Lake Minnetonka, MN

Lake Minnetonka, located about 15 miles south of Minneapolis, has been the site of several studies involving triclopyr. This highly developed region is a major recreational area. Eurasian Milfoil has grown to cover 600 to 1200ha of the lake since it was first detected in 1986 (Getsinger et al. 2000). The lake has a mean depth of 6.9 meters and a maximum depth of 30.8 metres. Summer temperatures for the region average 16.9 ^{o}C.

A 1994 aquatic dissipation study was conducted in three bays in Lake Minnetonka (Getsinger et al. 2000). Two 16 acre rectangular test plots, both dominated by Eurasian Milfoil, were treated with triclopyr triethanolamine (TEA) to achieve a concentration of 2.5 ppm, while the third plot served as a control. Water and sediment samples were collected at selected locations up to 1600m outside of the plots for six weeks post application. Dissipation half lives for triclopyr and TCP in water ranged from 3.7 to 4.7 days and from 4.2 to 7.9 days respectively with trace amounts of TMP found. Triclopyr rapidly degraded to its metabolites [DT 50: 3.7 days for an open bay (Phelps Bay) to 4.7 days for a bay with a restricted water inlet (Carson Bay)]. Light levels were low in the plots due to a dense submerged canopy of water millfoil growing in the test plots. The authors attribute the breakdown to microbial degradation.

[5] According to Anne Monnelly of the Massachusetts Department of Conservation and Recreation: in Massachusetts early Spring water temps can be expected to be at or near 4 ^{o}C . During the summer the temperature of the upper waters continues to climb, reaching a seasonal maximum usually in late July or August. This max can range from around 18-20 ^{o}C in a cold water lake to 25 ^{o}C in a shallow, warm water lake.

Trace levels of triclopyr (257 ppb) were found in sediment in the open Phelps Bay on day 3 of sampling which dissipated to below the limit of detection within four weeks (DT50, 5 days). In Carson Bay, the maximum level of triclopyr detected in sediment was 375 ppb on day 1 with subsequent dissipation to below the limit of detection by week 6.

The highest level of the TCP metabolite found in the Phelps Bay sediment was 27 ppb. In Carson Bay, TCP values peaked at 65 ppb by week 3. Half lives in sediment were 10.7 to 11.3 days.

The peak total residues (triclopyr and TCP) found in water at 400m intervals from the treated plots are shown below:

Distance from treatment area (metres)	Residue Level (ppb)
100	293.7
400	57.9
800	17.9
1600	12.5

Triclopyr metabolites were taken up, or "sequestered", by matrices such as sediment, fish, shellfish and plants. Dissipation from these matrices followed with a half life of between 2.5 and 13.7 days for triclopyr; 2.9 days 13.7 days for TCP; and 2.4 to 11.6 days for TMP. According to Getsinger, TMP levels in fish tissue were, unexpectedly, often two to four times the levels of the parent triclopyr. (Getsinger, 2000).

Whole Pond Studies

A whole pond treatment study was undertaken by Dow scientists with the Army Corps of Engineers in response to questions raised by EPA regarding triclopyr applications to an entire small water body (Foster, 1992). Man made, replicated outdoor ponds were selected in California, Missouri and Texas (3 at each site). Application was made to achieve a triclopyr concentration of 2.5 ppm in each pond in July (California), June (Missouri) and May (Texas). Water samples were collected at one third and two thirds of the total depth of the water column. Sediment samples were collected to a depth of 5 cm.

- ***California***
  In the California study, half lives of 6.9 and 7.5 days for triclopyr; 4.2 and 4.5 days for TCP and 5.3 and 7.7 days for TMP were calculated. In sediment, half lives of 3.4, 3.6 and 5.6 days were calculated for triclopyr, TMP and TCP respectively. Light intensity measurements showed that 65-90 % of surface light was quenched at depths greater than 75 cm in all ponds. Water from the test site ponds was characterized as "alkaline" with pH values of 7.8 to 8.1.

- ***Missouri***
  In the Missouri study, results were consistent with the California results with half lives of 5.9 and 6.1 days for triclopyr; 4.0 and 5.9 days for TCP and 4.0 and 4.8 days for TMP. In sediment, half lives of 2.8 and 3.2 days; 6.2 and 7 days, were calculated for triclopyr, and TMP respectively. Triclopyr levels were around 0.1ppm with TCP levels at 0.08ppm. Light intensity was quenched by approximately 50% in the upper 0.8 meters of the water column. Water pH values ranged from 7.9 to 9.4.

- ***Texas***

  Dissipation rates at the Texas site were consistent with the Missouri and California results with half lives in water of 6.5 days, 5.7 days and 6.5 days for triclopyr, TCP and TMP respectively. Sediment dissipation half lives were 4.6 days and 13.3 days for triclopyr and TCP respectively. Light intensity was quenched in the upper 0.5 meters by approximately 50%. The water pH values were around 8.

  A later Texas pond study shows that triclopyr dissipates rapidly from water with a half life of 6 days. TCP levels peaked at 5.5 ppb on day 14 and dissipated with a half life of 7.5 days. TMP Levels rose to ~ 4ppb and dissipated with a half-life of 8.8 days. In sediment, residues were low and dissipated with half lives of 4.5 to 5.6 days. No detectable residues of TMP metabolite were found in the samples.

Table 7 : Summary of half lives (days)in various water and sediment from Triclopyr Aquatic Dissipation Studies Dow Elanco. Study ID: GH-C 4526		Lake Seminole, GA, Aerial Plot	Lake Seminole, GA ,Boat Plot	Carsons Bay, L. Minnetonka, MN 1994	Phelps Bay, L. Minnetonka, MN 1994	Elkgrove, CA, Pond A 1995	Elkgrove, CA, Pond B 1995	Columbia, MO, Pond A 1995	Columbia, MO Pond B 1995	Lewisville, TX, Pond A 1995	Lewisville, TX, Pond B 1995	Lewisville, TX, 1996
Water	Triclopyr	3.5	0.5	4.7	3.7	6.9	7.5	5.9	6.1	6.5	6.3	6.0
	TCP			7.9	4.2	4.2	4.5	4.0	5.9	5.7	10.0	7.5
	TMP					5.3	7.7	4.0	4.8	6.5	5.7	8.8
Sediment	Triclopyr			5.8	5.0	3.4	3.6	2.8	3.2	4.6	4.6	4.5
	TCP			10.7	11.3	5.6	3.8	6.2	7.0	13.3	12.3	5.6

The half life values for triclopyr in the pond studies were longer than reported in Lake Minnetonka and Lake Seminole, probably due to the closed nature of the system.

Conclusion: Triclopyr TEA dissociates in water to the triclopyr acid which dissipates with half lives of between one and seven days due to photolysis, microbial action and dilution. While triclopyr is persistent in anaerobic aquatic environments, it is not found to persist in sediment in field dissipation studies. In shallow sediment, such as that in Lake Minnetonka**,** triclopyr dissipates with half lives of around 5 days. The metabolite TCP also dissipates quickly from both water and sediment.

3. Toxicity Profile

Carcinogenicity and Use of Triclopyr in Sensitive Areas

A thorough review of the toxicology and environmental fate database for triclopyr was completed by both MDAR and the Massachusetts Department of Environmental Protection (DEP) in 1991[6]. This review was conducted in order to evaluate the suitability of the triclopyr products, Garlon 3A and Garlon 4, for use in sensitive areas along rights-of-way (ROW). The review noted positive results in oncogenicity studies where there was an increase in the combined incidence of mammary adenomas and adenocarcinomas in female rats at high dose levels. Furthermore, under EPA's carcinogen classification scheme, triclopyr may be considered a group C carcinogen (possible human carcinogen: limited animal evidence).

As a result of the 1991 review, Garlon 4 (triclopyr BEE formulation) was recommended for inclusion on the ROW Sensitive Area Materials List. However, in an effort to limit the overall use of triclopyr while there remained outstanding carcinogenicity concerns, the placement of Garlon 3A (triclopyr TEA formulation) on the list was not supported.

DowElanco hired Pathco Inc., in an effort to seek additional review of the triclopyr oncogencity data by experts outside of their corporation. Pathco Inc. created a Pathology Working Group (PWG) to provide additional review of the rat and mouse tumor and pathology slides and data. The neoplasms examined included: mammary tumors (rats & mice), adrenal medullary pheochromocytomas, skin papillomas and subcutaneous fibromas (male rats). The PWG completed its review and issued their findings to DowElanco in 1996. According to the PWG, the weight-of-evidence indicates that the slight increase in mammary tumors was not related to triclopyr treatment in rats and mice; nor were the other tumors observed in rats and mice related to triclopyr treatment. The conclusion of the PWG's multi-volume report was that the overall weight of the evidence indicates that triclopry is not carcinogenic in either rats or mice (Goodman, p.24).

On August 9, 1995, the EPA's Carcinogenicity Peer Review Committee (CPRC), classified triclopyr as a Group D chemical (not classifiable as to human carcinogenicity). This decision was based on increases in mammary tumors in both the female rat and mouse, and adrenal pheochromocytomas in the male rat, which the majority of the CPRC believed to be only marginal. Overall the majority of the CPRC felt that the animal carcinogenicity evidence was marginal (not entirely negative, but yet not convincing). Therefore, the consensus of the CPRC was to classify triclopyr as a Group D chemical, based on what was considered only marginal response and the absence of additional support from structural analogs (e.g. chlorpyrifos)[7] or genotoxicity (1998 RED, p. 18).

[6] Copies of the 1991 MDAR and DEP ADHOC Committee Final Report are available upon request. Copies of the ROW Triclopry Factsheet are maintained on the following Department website: www.mass.gov/agr/pesticide/rightofway/index.htm

[7] TCP is a metabolite of triclopyr, chlopyrifos, and chlorpyrifos-methyl; therefore, chlorpyrifos is considered a structural analog of triclopyr. Its important to note that chlorpyrifos inhibits AchE and triclopyr does not. Toxicity studies of chlorpyrifos have not produced evidence of carcinogencity.

Toxicity and Exposure to Humans

A thorough review of the toxicology and environmental fate database was completed by both MDAR and MDEP in 1991 during the evaluation for determination of the acceptability of triclopyr for use in sensitive areas of right-of-ways (ROWs). Given that little new or additional mammalian toxicity data has been developed since that time, most of the summary data presented in this section is taken directly from the ROW Triclopyr Factsheet[8].

The Final Report concluded that the rat oral LD_{5O} for combined sexes has been reported as 713 mg/kg. Rabbits and guinea pigs have oral LD_{50} values of 550 and 310 mg/kg respectively. The target organ for triclopyr is in the liver. The only positive result in the oncogenicity studies was an increase in the combined incidence of mammary adenomas and adenocarcinoinas in the female rats at the high dose. Mutagenicity tests were negative. The developmental NOEL was reported as 75 mg/kg/d with a slight increase in maternal mortality.

Acute Toxicity

The results of acute toxicity studies conducted with triclopyr TEA formulation as provided below, indicate that the material is highly corrosive to the eyes and slightly toxic to relatively non-toxic via other routes of exposure (1998 RED, p. 7).

Study Type	Results	Toxicity Category
Acute Oral	LD_{50} = 1847 mg/kg (M+F)	III
Acute Dermal	LD_{50} >2000 mg/kg	III
Acute Inhalation	LC_{50} >2.6 mg/L	IV
Primary Eye Irritation	Corrosive	I
Primary Dermal Irritation	Not irritating	IV
Dermal Sensitization	sensitizer	N/A

Table 8: Acute Toxicity Categories Triclopyr TEA (44.4% a.i.)

Metabolism/Pharmacokinetics

Two studies, one dermal and one oral have been conducted in humans to determine pharmacokinetic and metabolic profiles. Five mg/kg acid equivalent (ae) was applied to the forearm of 5 volunteers in the dermal study. Results indicate that 1.58% to 1.11% of the applied dose was absorbed and the percutaneous absorption half -life was 16.8 hours . In the oral study, 6 volunteers received 0.1 or 0.5 mg/kg Triclopyr (acid equivalent) in apple juice. The excretion half—life is 5 hours and 80% of the dose is recovered as unchanged Triclopyr in the urine. The 20% which was unaccounted for could be attributed to one of several explanations including incomplete collections of urine, incomplete absorption of material or metabolism to an unknown metabolite (1991 MDAR and DEP ADHOC Committee Report, p.4).

[8] A Review of the Herbicide Triclopyr Pursuant to 333 CMR 12.04(1)(d), February, 1991 as developed by MDAR and DEP ADHOC Committee.

Bioequivalency for the Purpose of Testing the Three Chemical Forms of Triclopyr
Numerous toxicology studies have been conducted with triclopyr using either the parent compound (free acid), triethylamine salt (TEA), or the butoxyethyl ester (BEE) form of triclopyr. The issue of bioequivalency for the purpose of testing the three chemical forms of triclopyr (acid,triethylamine salt, and butoxyethyl ester) was addressed by the registrant conducting special studies with the triethylamine and butoxyethyl ester forms of triclopyr. These studies, which included data on comparative disposition, plasma half-life, tissue distribution, hydrolytic cleavage under physiological and environmental conditions for triclopyr TEA and triclopyr BEE were found to adequately address the issue of bioequivalency. In addition, subchronic toxicity studies conducted with each form supported the pharmacokinetic data in demonstrating bioequivalence. Therefore, with the exception of the acute toxicity database (where differences in Toxicity Categories have been noted above), studies conducted with any one form of triclopyr have been used to support the toxicology database as a whole (1998 RED, p.8).

EPA Reregistration Post FQPA

The registration of the Renovate 3 uses, in ponds, lakes, reservoirs, and in non-irrigation canals or ditches, was granted after the completion of the 1998 US EPA RED and thus, it does not consider the new uses. The RED does consider uses on rice, rangeland and pasture, rights-of-way, forestry and turf, including home lawns, for control of broadleaf weeds and woody plants.

At the time of re-registration there were 12-registered products containing triclopyr butoxyethyl ester (BEE) and 24-products containing triclopyr triethylamine salt (TEA). The Agency determined that all uses, when labeled and used as specified in the RED, were eligible for re-registration. The 1998 RED considered the requirements of the "Food Quality Protection Act of 1996" (FQPA) including the following when establishing or reassessing tolerances:

- Aggregate exposures to pesticide residues, including all anticipated dietary exposures and other exposures for which there is reliable information;
- Cumulative effects from a pesticide and other compounds with a common mechanism of toxicity;
- Susceptibility of infants and children to the toxic effects of pesticide residues; and
- Endocrine disrupting effects.

The 1998 RED considered only dietary and drinking water exposure in the aggregate assessment, since other non-occupational exposures to triclopyr were expected to be minimal. Calculations using the existing triclopyr tolerances resulted in a theoretical maximum residue concentration (TMRC), which represented <1% of the RfD for the general population and < 3% of the RfD for children less than one year old, considering food only.

EPA established the RfD for triclopyr at 0.05 mg/kg/day based on a reproductive toxicity study in rats with a NOEL of 5 mg/kg/day using an Uncertainty Factor of 100. At the next higher dose level (HDL) of 25 mg/kg/day, an increased incidence of degeneration of the proximal tubules of the kidney was observed in P_1 and P_2 parents of both sexes (Federal Register / Vol. 62, No. 172 / Friday, September 5, 1997 / Rules and Regulations).

Dietary Exposure from Use of Renovate 3
The application restrictions warnings and prohibitions found in the Renovate 3 labeling and discussed previously, in the General Directions and Restrictions section of this review, limit exposure to treated waters.

Renovate 3 is not registered for use on any agricultural commodities. Although the labeling does not bear any restrictions on fishing or livestock consumption of water from the treatment area, no significant contributions to dietary exposure are expected from the use of Renovate 3.

The following product label prohibitions mitigate much of the potential for additional residues of triclopyr in or on agricultural commodities:

- Prohibition for chemigation or application via any irrigation system;
- Prohibition on applications where runoff water may flow onto agricultural land; and
- Prohibition on the use of treated water for irrigation until 120-days following application or until non-detectable by laboratory analysis (immunoassay).

Triclopyr tolerances are established for the combined residues of the parent triclopyr acid [((3,5,6-trichloro-2-pyridinyl)oxy)acetic acid] and its metabolites TCP [3,5,6-trichloro-2-pyridinol] and TMP [3,5,6-trichloro-2-methoxypyridine] in or on the following raw agricultural commodities:[9]

Commodity	Parts per million (ppm)
Grasses, forage	500
Grasses	forage, hay 500
Fish	0.2
Shellfish	5.0

Table 9: Maximum Allowable Residues of Triclopry Acid, and its Metabolites TCP and TMP

Tolerances are also established for the combined residues of only the parent triclopyr acid [((3,5,6-trichloro- 2-pyridinyl)oxy)acetic acid] and its metabolite TCP [3,5,6-trichloro-2-pyridinol] in or on the following raw agricultural commodities:

Commodity	Parts per million (ppm)
Eggs	0.05
Meat, fat, and meat byproducts (except liver and kidney) of cattle, goats, hogs, horses, and sheep	0.05
Meat, fat, and meat byproducts (except kidney) of poultry	0.1
Milk	0.01
Liver and kidney of cattle, goats, hogs, horses, and sheep	0.5
Rice, grain	0.3
Rice, straw	10.0

Table 10: Maximum Allowable Residues of Triclopry Acid and the Metabolite TCP

[9] 40 CFR § 180.417 Triclopyr; tolerances for residues and Federal Register / Vol. 67, No. 181 / Wednesday, September 18, 2002 / Rules and Regulations.

Exposure from Drinking Water and Recreational Uses of Treated Water

The Renovate 3 product labeling does not bear any restrictions on use of water in the treatment area for recreational purposes, including swimming and fishing. Given that triclopyr residues in water degrade rapidly via photolysis, the risks from exposure to triclopyr via drinking water or recreational uses should be negligible based on the following:

- That triclopyr is slightly toxic via acute oral and dermal route of exposure and is not a dermal sensitizer;
- That triclopyr use in waters of the Commonwealth, used for drinking water and recreational purposes, are highly regulated and expected to result in intermittent exposures to those using such waters;
- That triclopyr EEC in treated water from maximum label application rates is 2.5 ppm;
- That Renovate 3 labeling requires minimum setback distances from functioning potable water intakes (see: Table 4: Minimum Setback Distances from Functioning Potable Water Intakes):
- That Renovate 3 labeling requires that in order to make applications around and within the distances noted in the Table 4: Minimum Setback Distances from Functioning Potable Water Intakes, that functioning potable water intakes be turned off until the triclopyr level in the intake water is determined to be 0.4 ppm or less by laboratory analysis or immunoassay;

When the EPA established the tolerance for combined residues of triclopyr and its metabolites, TCP and TMP in or on fish at 0.2 ppm and shellfish at 5.0 ppm, it conducted a comprehensive risk assessment using modeling and risk assessment techniques to estimate maximum exposure potential from all sources (total aggregate exposure) including food, drinking water, and residential uses. This risk assessment concluded that there is a reasonable certainty that no harm will result to the general population, and to infants and children from aggregate exposure to triclopyr and TCP (Federal Register / Vol. 67, No. 181 / Wednesday, September 18, 2002 / Rules and Regulations).

4. Ecotoxicity[10]

As stated earlier, the application restrictions warnings and prohibitions found in the Renovate 3 labeling and discussed previously, in the General Directions and Restrictions section of this review, limit exposure to certain wildlife from treated waters. These labeling prohibitions include the following:

- Do not apply to salt water bays or estuaries;
- Do not apply directly to un-impounded rivers or streams; and
- When making applications to control unwanted plants on banks or shorelines of moving water sites, minimize over-spray to open water.

It should also be noted that due to the relatively low toxicity of triclopyr TEA to wildlife, the Environmental Hazards section of this labeling does not include precautionary label statements relative to toxicity to aquatic invertebrates, fish, or waterfowl.

Threatened and Endangered Species

The Renovate product labeling includes only a very brief discussion relative to applications near sensitive areas. The labeling states that pesticide should only be applied when the potential for drift to adjacent sensitive areas (e.g.. residential areas, known habitat for threatened or endangered species, non-target crops) is minimal (e.g., when wind is blowing away from the sensitive areas).

As discussed in the Peterson, 1994 study in the Plant Toxicity section of this review there are considerable differences in sensitivity among plant species and use of uncertainty factors is necessary to provide an acceptable margin of safety in evaluating the hazard presented by herbicides to the aquatic environment. There are a variety of plants and animals such as certain amphibian spp., bladderwort spp., water-milfoil spp. pondweed spp. etc. that may be harmed by applications of Renovate 3 in Massachusetts's lakes and ponds.

According to the Massachusetts Natural Heritage and Endangered Species Program (NHESP) in Westborough, there are 258 species of native plants that are officially listed as endangered, threatened or of special concern in Massachusetts and tracked by the NHESP.[11] Massachusetts has laws, regulations, and processes in place such that potential impacts on T&E species may be mitigated via site-specific conditions or requirements, such as setbacks or other application restrictions that may be employed through the permitting process.

The Massachusetts Wetlands Protection Act (M.G.L. c.131, s.40 and regulations 310 CMR 10.00) requires that proposed alterations to the wetland habitats of rare wildlife be reviewed by the NHESP. Alterations that would have short or long term adverse effects on the wetland habitats of rare wildlife species are prohibited.

A Notice of Intent (NOI) must be sent to the Conservation Commission with a copy to the Department of Environmental Protection Regional Office. If the proposed project occurs within an Estimated Habitat of Rare Wildlife in the most recent version of the Natural Heritage Atlas, a copy of the Notice of Intent must be submitted to the NHESP. If the proposed project occurs

[10] Note that many toxicity studies, especially field studies, use salt (TEA) or ester (BEE) formulations; however, unless otherwise indicated, it is the parent acid equivalent that is reported for concentrations used or detected in treated medium.

[11] NHESP maintains lists of these species and related data on the following website: http://www.mass.gov/dfwele/dfw/nhesp/nhrare.htm

within a Priority Habitat of Rare Species in the most recent version of the Natural Heritage Atlas, the project proponent must submit project plans to the NHESP for an impact determination. An Order of Conditions must be obtained from the Conservation Commission prior to work (2004 GEIR, p. 4-125).

Amphibian Toxicity

FETAX with Garlon 3A and Garlon 4

The toxicity of Garlon 3A and Garlon 4 on embryonic development was examined and compared using the 96-hr static renewal, whole embryo assay for identifying teratogenic and developmental toxicants-Frog Embryo Teratogenesis Assay-Xenopus (FETAX). Garlon 3A had an LC_5 and LC_{50} of 119 and 162.5 mg/L, respectively and Garlon 4 had an LC_5 and LC_{50} of 6.7 and 9.3 mg/L, respectively. Thus when the highest rates recommended for triclopyr (see table below) are applied to water 15 cm in depth, the EEC calculated on the basis of acid equivalent would be ~2.5 mg/L, respectively. The margins of exposure (MOE) derived as per the LC_5[12] divided by the EEC for frog embryos exposed to these concentrations would be approximately 2 and 47, for Garlon 4 and Garlon 3A respectively. Based on the above acute toxicity endpoints, Garlon 4 (triclopyr BEE) shows significantly greater toxicity to *X. Laevis* embryos as compared to Garlon 3 (triclopyr TEA) (Perkins, 2000).

Treatment	**Highest recommended application rate (L/ha)**	**EEC in water 15 cm in depth**	**Margin of Exposure (MOE)[13] (LC_5/EEC)**	**Risk Quotient[14] (EEC/LC_{50})**
Garlon 3A	10.72	~2.5 mg ae/L	~47	~0.016
Garlon 4	8	~2.5 mg ae/L	~2.7	~0.269

Table 11: Margin of Safety Calculation for Triclopyr Applied at the Highest Recommended Application Rates to Water 15 cm in depth.

[12] For the purposes of calculating the MOE, a minimally acute level, such as the LC_5 is sometimes substituted for a determined NOEL or NOAEL.

[13] The Margin of Exposure (MOE) is a measure of how close the high-end exposure comes to the NOEL (the highest dose at which no effects were observed in the laboratory test), and is calculated as the ratio of the NOEL to the exposure (NOEL/exposure = MOE). The MOE is calculated using the EEC from nominal application rates. According to the product labeling and assuming uniform mixing, as calculated in Appendix C. of this report, 2.5 mg/L (ppm) is the Expected Environmental Concentrations (EEC) from the application of Renovate 3 at 2.3 gal. Renovate /acre water that is 1-ft. deep.

[14] Risk Quotient (RQ): The estimated environmental concentration (EEC) divided by the median lethal dose (LC_{50}); The lower the Risk Quotient (RQ) the less risk. According to EPA's methodology, if the quotient exceeds the value 1, then a significant risk may be indicated.

FETAX and Larval Toxicity Study with Varying pH and Release® (triclopyr BEE)
In an amphibian embryonic and larval laboratory toxicity study, an evaluation and comparison were made relative to sensitivity to the combination of pH and Release® (triclopyr BEE[15]) concentration. As part of this work an interspecies comparison of sensitivity was conducted using the same four anuran species, *Rana pipiens, Rana clamitans, Bufo americanus,* and *Xenopus laevis.* The FETAX test was employed for embryo testing and American Society for Testing and Materials (ASTM) guidelines were employed for larvae testing. The amphibians were exposed to treatments for at least 96-hr in a static renewal system using combinations of five levels for both pH (4.5, 5.0, 6.5, 8.0, and 8.5) and Release® concentrations ranging from 0.1 to 25 mg/L triclopyr (individual dose values not provided).

Observations and data indicated a trend of increased Release® toxicity under low pH conditions and in all comparisons based on LC_{10} and LC_{50} estimates, irrespective of species or life stage. The triclopyr BEE median lethal concentrations (LC_{50}) for *X. laevis* embryos were 8.3 mg/L and 13.7 mg/L at pH 5.5 and 7 respectively. These values are in relative agreement with the results from the Perkins et.al. study described previously. Larval lethal concentration estimates were eight to twenty-three times less than those observed for embryos, indicating that the larval stages were more sensitive to treatments. The cause(s) for this increase sensitivity are not well understood, but may be due to target organ formation or increase metabolic activity of post-embryonic life-stages. The median lethal concentration (LC_{50}) values for the larvae were below the Release® EEC of 2.7 ppm.

Species sensitivity was similar, with an average larval 96-h LC_{50} of 0.89 mg/L at pH 5.5 and 1.6 mg/L triclopyr at pH 7. For the embryo tests, *R. pipiens* were slightly less sensitive in comparison with the other three species. *R. pipiens* and *X. laevis* had malformations (abnormal gut coiling) above that observed in controls. For *X. laevis* embryos, the 96-hr EC_{50} values for malformations were 13.2 mg/L (11.1, 17.6) and 14.8 mg/L (13.1, 19.9) for pH 5.5 and 7, respectively. Using the corresponding embryo LC_{50} values in the table below, the corresponding *X. laevis* Teratogenic Index at pH 5.5 and 7 was 0.62 and 0.93, respectively. The teratogenic index was measured by dividing the LC_{50} by the EC_{50} and served as a measure of relative teratogenicity. According to the ASTM, when the teratogenic index is greater than 1.5, the test substance is classified as a suspect teratogen. Based on the above information for *R. pipiens* and *X. laevis*, the combination of Release® and pH was not deemed to be teratogenic. No malformations were observed in the larval tests. These results follow a notable trend throughout the available triclopyr ecotoxicological database, whereby tested species show much greater sensitivity to triclopyr BEE as compared to studies using triclopyr TEA formulation (Edginton, 2003).

[15] The formulated triclopyr product Release® was used in all tests. Both Release® and Garlon® 4 contain the same formulation of triclopyr BEE and inert or "other ingredients" and differ only in their registered uses.

Species	Life Stage	PH	96-hr LC_{10} (mg/L) (95% confidence intervals)	96-hr LC_{50} (mg/L) (95% confidence intervals)	Risk Quotient [16] (EEC/LC_{50})
Bufo americanus	Embryo	5.5	7.4 (0.62, 8.9)	12.0 (10.0, 14.4)	0.2
		7	9.5 (6.1, 11.6)	15.1 (13.4, 18.4)	0.2
	Larvae	5.5	0.60 (0.50, 0.66)	0.88 (0.78, 0.99)	3.1
		7	1.1 (0.95, 1.6)	2.1 (1.6, 7.0)	1.3
Rana pipiens	Embryo	5.5	9.2 (7.7, 10.5)	16.2 (14.8, 17.7)	0.2
		7	14.1 (12.6, 15.9)	23.3 (20.6, 28.4)	0.1
	Larvae	5.5	0.66 (0.64, 0.70)	0.79 (0.75, 0.93)	3.4
		7	0.68 (0.65, 1.4)	0.87 (0.73, 1.0)	3.1
Rana clamitans,	Embryo 96-hr	5.5	5.9 (3.5, 8.9)	19.0 (16.0, 27.8)	0.1
		7	11.6 (8.8, 14.0)	24.6 (20.1, 44.9)	0.1
	Embryo 7-day	5.5	6.9 (4.1, 8.2)	11.5 (10.1, 13.2)	0.2
		7	10.8 (8.9, 12.6)	18.2 (16.1, 21.7)	0.1
Xenopus laevis	Embryo	5.5	4.8 (3.9, 5.6)	8.3 (7.6, 8.9)	0.3
		7	6.7 (5.2, 7.8)	13.7 (12.4, 16.1)	0.2
	Larvae	5.5	0.34 (0.16, 0.51)	1.0 (0.75, 1.2)	2.7
		7	0.59 (0.35, 0.79)	1.7 (1.4, 2.1)	1.6

Table 12: Comparitive Toxicity of Release® (a.i. triclopyr BEE) to Four Amphibian Species at pH 5.5 and pH 7.0.

[16] Risk Quotient (RQ): The estimated environmental concentration (EEC) used in this study would be 2.7 mg ae/L triclopyr divided by the median lethal dose (LC_{50}). The lower the Risk Quotient (RQ) the less risk. According to EPA's methodology, if the quotient exceeds the value 1, then a significant risk may be indicated.

Freshwater Invertebrates Toxicity

The triclopyr review completed by the MDAR and DEP ADHOC Committee in 1991 included data from the USDA Forest Service Agriculture Handbook #633 Vol. 1, Pesticide Background Statements: Aug. 1984. This data indicated low acute lethal toxicity here to organisms tested, with a 48-hr LC_{5O} for Daphnids reported as 1,170 ppm[17] triclopyr.

Waterflea Acute Toxicity Study with Triclopyr Acid

As might be expected, the endpoints for acute toxicity testing using technical grade active ingredient (TGAI) report much higher toxicity as compared to the TEA end-use products. Nonetheless, the results of submitted studies indicate that TGAI triclopyr acid is practically non-toxic to freshwater invertebrates. Results of this test are provided below (EPA RED 1998, p.43).

Species	% AI	LC_{50} or EC_{50} (ppm)	Toxicity Category	Risk Quotient[18] (EEC/LC_{50})[19]
Waterflea (Daphnia Magna)	99.5	132.9	Practically non-toxic	~0.018

Table 13: Aquatic Invertebrate Toxicity - Tricloppyr Acid

Waterflea Acute Toxicity Study with Triclopyr TEA

Testing of triclopyr TEA end-use-products (EUP) indicate much lower toxicity than TGAI. The triclopyr TEA EUP is practically non-toxic to aquatic invertebrates on an acute basis.

Species	% AI	LC_{50} or EC_{50} (ppm)	Toxicity Category	Risk Quotient (EEC/LC_{50})
Waterflea (Daphnia Magna)	44.9	1,496	Practically non-toxic	~0.00167

Table 14: Aquatic Invertebrate Toxicity - Triclopyr TEA

Waterflea Life-Cycle Toxicity Study with Triclopyr TEA

In another study the acute and chronic toxicity of triclopyr TEA was determined for the freshwater invertebrate, water flea (*Daphnia magna* Straus)[20]. The acute test consisted of exposing groups of 10 neonates to six concentrations (336, 480, 636, 980, 1400 and 2000 mg/L) of the test material, triclopyr TEA salt, and a control. The calculated 48-hr LC_{50} value for triclopyr, based on nominal concentrations, was 1,170 (1,030 – 1,340) mg/L. In the 21-day

[17] These data agree with the results from the 1984 Gersich study (found below) using formulation triclopyr TEA at 44.9%.

[18] Risk Quotient (RQ): The estimated environmental concentration (EEC) divided by the median lethal dose (LC50); The lower the Risk Quotient (RQ) the less risk. According to EPA's methodology, if the quotient exceeds the value 1, then a significant risk may be indicated.

[19] According to the product labeling and assuming uniform mixing, as calculated in Appendix C. of this report, 2.5 mg/L (ppm) is the Expected Environmental Concentrations (EEC) from the application of Renovate 3 at 2.3 gal. Renovate /acre water that is 1-ft. deep.

[20] Water fleas are crustaceans from the **order Cladocera** that are sometimes extremely abundant in freshwater pools. They appear in high concentrations in pools, ponds, lakes, ditches, slow-moving streams, and swamps where organic material is decomposing and are ideally suited for feeding freshwater fish fry.

chronic toxicity testing a static renewal procedure was used whereby there was a batchwise replacement of test and control solutions at regular intervals. Triclopyr TEA salt concentrations used for the chronic test were 80.7, 149, 290, 574 and 1,177 mg/L with four replicates for each test concentration and the control, resulting in five daphnids/replicate or a total of 20 organisms per concentration. The 21-day chronic toxicity LC_{50} value, based on analyzed concentrations was 1,140 (950 - 1590) mg/L. The chronic data were used to estimate the Maximum Acceptable Toxicant Concentration (MATC)[21]. The authors state that the MATC lies between 80.7 and 149.0 mg/L and may be expressed as the geometric mean of 80.7 and 149.0, or 110 mg/L. The estimation of the MATC was based on data associated with the reproductive endpoints, mean total young/daphnid and mean brood size. These two endpoints both significantly differed from the control at the 149.0 mg/L level. (Gersich, 1984).

Species	% A.I.	NOEC/LOEC (ppm)	MATC (ppm)	Endpoints Affected
Daphnid *(Daphnia magna)*	44.9	NOEC 80.7 LOEC 149.0	110	Total young and mean brood size

Table 15: Freshwater Aquatic Invertebrate Life Cycle Toxicity†

† Table as printed in the 1998 EPA RED, p. 44.

Plankton Toxicity

The effects of triclopyr and other common aerially applied rice herbicides on plankton communities of aquaculture fish ponds were studied at the University of Arkansas at Pine Bluff, Aquaculture Research Station. Parameters examined include phytoplankton biomass and productivity, zooplankton populations, and critical water quality variables of morning dissolved oxygen, water temperature, pH, total ammonia nitrogen, nitrite nitrogen, and chlorophyll. Grandstand Herbicide[22] was applied in four treatments to four 550-liter outdoor pool mesocosms each was designed to evaluate simulated direct rate over-spraying of ponds, drift at 1% and 10% of direct rates, and an untreated control. The triclopyr rate of application was 0.4 kg a.i./ha (0.357 lb a.i./A). No measurable impact was detected from any compound on any water quality or plankton variable (Perschbacher,2002).

Other Freshwater Invertebrate Toxicity Studies Using Triclopyr BEE

[Although the researchers of the following two studies used the triclopyr BEE formulation, the data are included in this review of Renovate 3, a.i. triclopry TEA, given the relatively few published non-target freshwater invertebrate studies available.]

A New Zealand study investigated the impact of an application of triclopyr in the Ahuriri River on aquatic benthic macroinvertebrates, following a large-scale application of Grazon Herbicide[23] made via helicopter. Species abundance and composition of aquatic benthic macroinvertebrates were compared between an area treated with triclopyr, and an untreated upstream control site.

[21] MATC: The hypothetical toxic threshold concentration lying in a range bounded at the lower by the highest tested concentration having no observed effect (NOEC) and at the high end by the lowest concentration having a significant toxic effect (LOEC) in a life cycle (full chronic) or partial life cycle (partial chronic) test. This may be represented as NOEC < MATC < LOEC. Calculation of MATC requires quantitative life cycle toxicity data on the effects of a material on survival, growth, and reproduction.

[22] Grandstand Herbicide, EPA Reg. No. 62719-215 is registered for use in rice and is formulated similar to Garlon 3A/Renovate 3 with 44.4% triclopyr TEA, 31.8% triclopyr acid equivalent (ae), and 3 lb. a.i. /gallon.

[23] Grazon is sold in New Zealand and contains 600 g/litre triclopyr BEE. www.dowagro.com/nz/prod/herb.htm.

The site was described as having significant importance for native wildlife, including the endangered and threatened avian species which breed within the boundaries of the active river channel. The aquatic invertebrate species composition was similar in treatment and control sites, and did not change over time. The five taxa that made up 91-95% of all invertebrates by abundance did not vary significantly in treatment compared to control riffles[24] (Maloney, 1995).

As part of an assessment of the risks of adverse effects to aquatic organisms via runoff, aerial drift, or inadvertent overspray, flow-through toxicity tests were conducted to determine the effects of exposure time on the toxicity of triclopyr BEE (Garlon 4)[25] to stream insects caddisflies (*Hydropsyche* sp.[26]) and mayflies (*Isonychia* sp.[27]). The toxicity of triclopyr BEE to aquatic insects increased with increasing exposure time. There was no significant mortality of insects following 3-h exposures to the maximum test concentration of approximately 110 mg/L. Median lethal concentrations following 9- and 24-hr exposures were 14.9 and 4.0 mg/L for *Hydropsyche* sp., and 37.0 and 8.8 mg/L for *Isonychia* sp. respectively. The actual concentrations to which the insects were exposed are not know as residue analyses were not available due to limited resources allocated to the project. There is likely to be low risks to aquatic insects from use of Renovate 3 given that the triclopyr BEE formulations are more highly toxic to aquatic organisms as compared to the triclopyr TEA formulations (Garlon 3A/Renovate 3). The risk quotient values calculated in the table below also indicate minimal risk (Kreutzweiser, 1994).

Species	**Exposure Time**	**LC_{50} (ppm)**	**Toxicity Category**	**Risk Quotient[28] (EEC[29] / LC_{50})**
Caddisflies (*Hydropsyche* sp.)	9-hr	14.9	Slightly toxic	~0.17
	24-hr	4.0	Moderately Toxic	~0.63
Mayflies (*Isonychia* sp.)	9-hr	37.0	Slightly Toxic	~0.07
	24-hr	8.8	Moderately Toxic	~0.28

Table 16: Risk Quotients for Caddisflies and Mayflies Exposed to Garlon 4 (44.3% triclopyr BEE)

[24] A riffle is a segment of the river where the flow is shallower and more turbulent.
[25] Garlon 4, 44.3% a.i triclopyr BEE is not registered for use in aquatic weed management. It has been registered for use in forest vegetation management in the U Ssince 1979 and ground applications in Canadian forestry since 1991.
[26] *Trichoptera* : *Hydropsychidae* are commonly known as Caddisflies. Feeding primarily occurs in the larval stage of growth where they are found in rivers, streams and shallow pools.
[27] *Ephemeroptera: Isonychiidae* are commonly known as mayflies. Larvae are the only feeding stage, the adults have no functional gut. Like caddisflies, the larvae have gills and require unpolluted, well oxygenated, cool water to survive and are valuable tools for monitoring organic and chemical contamination of habitats.
[28] Risk Quotient (RQ): The estimated environmental concentration (EEC) divided by the median lethal dose (LC50); The lower the Risk Quotient (RQ) the less risk. According to EPA's methodology, if the quotient exceeds the value 1, then a significant risk may be indicated.
[29] Assuming uniform mixing, as shown in Appendix C., 2.5 mg/L (ppm) is the Expected Environmental Concentrations (EEC) from the application of Renovate 3 at 2.3 gal. Renovate /acre of water that is 1-ft. deep.

Estuarine and Marine Animal Toxicity

The Renovate 3 product labeling prohibits application to salt water or estuaries. The following information is provided given that estuaries might receive highly diluted treated water.

The triclopyr review completed by the MDAR and DEP ADHOC Committee in 1991 indicated low acute lethal toxicity[30] to organisms tested, with a 96-hr LC_{5O} of 895 ppm in shrimp, 96-hr LC_{5O} greater than 1,000 ppm in crabs, and 48-hr LC_{5O}s ranging between 56 and 87 ppm in oysters.

According to the 1998 EPA RED, estuarine/marine acute toxicity studies the fiddler crab and pink shrimp studies were graded as supplemental and did not fulfill EPA guidelines; however, the both Eastern oyster, Grass shrimp, and Tidewater silverside studies were found to fulfill the core data requirements and study guidelines. The results indicate that triclopyr TEA is slightly toxic to practically non-toxic to estuarine/marine invertebrates on an acute basis and practically non-toxic to estuarine/marine fish on an acute basis. The results of the studies are provided below

Species	**% A.I.**	**$LC_{50}/EC_{5(1}$ (ppm)**	**Toxicity Category**
Eastern oyster (shell deposition) *(Crassostrea virginica)*	46.09	58	Slightly
Eastern oyster (embryo-larvae) *(Crassostrea virginica)*	43.8	>56 <87ppm (48 hr EC_{50})	100% abnormal development at 87 ppm
Fiddler crab *(Uca pugilator)*	43.8	>1000	practically non-toxic
Grass shrimp *(Palaemontes pugio)*	46.09	326	practically non-toxic
Pink shrimp *(Penaeus duorarum)*	43.8	895	practically non-toxic
Tidewater silverside *(Menidia beryllina)*	44.7	130	practically non-toxic

Table 17: Estuarine/Marine Acute Toxicity- Triclopyr TEA

[30] Acute LC_{50} Freshwater Invertebrates; EPA Guidelines 72-2: In aquatic organisms, LC_{5O}s greater than 10 ppm are considered to be indicative of only slight toxicity and LC_{5O}s less than 1 ppm are considered to reflect high acute toxicity.

Fish Toxicity

Overview of Dissolved Oxygen-Related Fish Kills and Product Label Restrictions

Fish kills commonly result from reduced levels of dissolved oxygen (DO) in water. Reductions in DO may be caused by a number of natural events, such as a die-off of the microscopic green plants (phytoplankton) in the pond, or overturns in which oxygen deficient water from the deeper levels of the pond mixes with water in the upper levels.

As is discussed in the 2004 GEIR, the use of physical, mechanical, or chemical controls to help manage excessive aquatic plant growth in eutrophic waters results in direct and indirect effects on water quality such water transparency, biological oxygen demand, and dissolved oxygen. Such affects can significantly contribute to fish suffocation, especially during the summer months when elevated water temperatures reduce the capacity of water to retain oxygen.

The Environmental Hazards section of the labeling attempts to address the potential for depleted oxygen and the related increased biological oxygen demand (BOD) as per the increased decomposition of treated plants by bacteria and fungi. The labeling states that applicators should not treat more than one-third to one-half of the water area in a single operation and wait at least 10- to 14-days between treatments. The risks of a direct triclopyr induced phytoplankton die-off would appear to be minimal, based on data in the plant toxicity section of this review and highlighted in **Table 27** (p. 36) which show minimal risk from EEC of triclopry to freshwater green algae (*Selenastrum capricornutum*) (Peterson, 1994).

As required by product labeling, applications must begin along the shore and proceed outwards in bands to allow fish to move into untreated areas. Applications for control of unwanted plants on banks or shorelines of moving water sites must be made to minimize overspray to open water.

Summary of Fish Toxicity Data

Overall, the laboratory data below indicate that labeled applications of Renovate 3 should have minimal impact on fish in Massachusetts; however, the laboratory testing limits the complexity of effects on other parameters as is seen with dissolved oxygen.

The triclopyr review completed by the MDAR and DEP ADHOC Committee in 1991 stated that triclopyr TEA is "slightly toxic" to fish with 96-hr LC_{5O} values of 552 and 891 ppm for rainbow trout and bluegills respectively. The corresponding values for unformulated triclopyr (parent compound) are 117 ppm for rainbow trout and 148 ppm for bluegill. Both fish species were less sensitive to Garlon 3A than to the parent compound.

Acute Physiological Stress Response of Juvenile Coho Salmon to Sublethal Concentrations of Garlon 3A® and Garlon 4®

Juvenile coho salmon (*Oncorhynchus kisutch*) were exposed for 4-hr to sublethal concentrations of triclopyr. The nominal concentrations were 0.12, 0.24, 1.2, and 1.92 ppm for the herbicide Garlon 4® and 20, 40, 200, and 320 ppm for the herbicide Garlon 3A®. Trials were performed in a closed-system respirometer that measured oxygen consumption of fish prior to and during exposure. The water supply was maintained at pH 6.1 to 6.3 and 95% saturated with O_2. At the end of the exposure period, plasma glucose and lactate concentrations, hematocrit, and leucocrit were measured as indicators of acute physiological stress and compared to controls. There were no biologically significant indications of acute physiological stress in fish exposed to either formulation 5% to 80% of the 96-hr LC_{50} values. The concentrations used were based on

median lethal concentration for juvenile rainbow trout (*Salmo gairdneri)* at 2.4 and 400 ppm for Garlon 4 and Garlon 3A, respectively (Janz, 1991).

Bioconcentration in Fish

In an experiment to determine the levels and identity of [^{14}C] residues in bluegills (*Lepomis macrochirus*) exposed to 2.5 mg/L triclopyr TEA, the highest level of radioactivity observed in the flesh of fish (edible portion) at any time point was 0.13 mg/kg, calculated as acid equivalent. This level is less than 5% of the fish exposure level. The maximum level in the remainder (head, skin, and viscera) was about 95% (2.33 mg/kg) of the fish exposure level, indicating no concentrating effect. The principal components observed in the fish tissues were the parent acid triclopyr, 3,5,6-trichloro-2-pyridinol, 2-methoxy-3-,5,6-trichloropyridine and a conjugate. These components accounted for greater than 75% of all the residues observed. Bluegills exposed to [^{14}C] triclopyr under static conditions in an aquarium had a very low concentration factor, with a fish flesh (edible portion) factor (C_f/C_w) of 0.03 and a whole fish (C_f/C_w) factor of about 0.5 at apparent steady state. The exposure time to reach steady state was short, with an estimate of less than 1 day for fish flesh and 2 to 4 days for whole fish (Lickly, 1987).

Based on the 1998 EPA RED, triclopyr acid is practically non-toxic to freshwater fish on an acute basis. The related endpoint values and toxicity categories are provided below(p. 40):

Species	% A.I.	LC_{50} (ppm)	Toxicity Category
Rainbow trout *(Oncorhynchus mykiss)*	Technical	117	practically non- toxic
Bluegill sunfish *(Lepomismacrochirus)*	Technical	148	practically non- toxic

Table 18: Freshwater Fish Acute Toxicity with Triclopyr Acid

The RED also indicates that triclopyr TEA is practically non-toxic to freshwater fish on an acute basis. The related endpoint values and toxicity categories are provided below:

Species	% A.I.	LC_{50} (ppm)	Toxicity Category
Rainbow trout *(Oncorhynchus my kiss)*	64.7	613 (flow-through)	practically non-toxic
Rainbow trout *(On corhynch usmy kiss)*	47.8	240 (flow-through)	practically non-toxic
Bluegill sunfish *(Lepomis macrochirus)*	64.7	893 (flow-through)	practically non-toxic
Bluegill sunfish *(Lepomis macrochirus)*	47.8	471 (flow-through)	practically non-toxic
Fathead minnow *(Pimephales promelas)*	64.7	947 (flow-through)	practically non-toxic
Fathead minnow *(Pimephales promelas)*	44.9	544 (static)	practically non-toxic
Fathead minnow *(Pimephales promelas)*	44.9	279 (flow-through)	practically non-toxic

Table 19: Freshwater Fish Acute Toxicity with Triclopyr TEA

As described graphically in Appendix A, and according to the environmental fate review, the parent compound (triclopyr acid) is short-lived in the aquatic environment with reported field dissipation half-lives from 0.5 days to 7.5 days. The principal decay product of the acid is 3,5,6-trichloro-2-pyridinol (TCP), a transient metabolite in water with field dissipation half-lives ranging from 4.2 days to 10 days [see: Field Dissipation Studies (p.13)]. TCP degrades into nonhalagenated, low molecular weight organic acids with phototransformation playing the primary role in this process.

As per the 1998 EPA RED, the acute toxicity of TCP to freshwater fish is provided in the table below:

Species	**%AI**	**LC_{50} (ppm)**	**Toxicity Category**
Bluegill sunfish	99.9%	12.5	slightly toxic
Rainbow trout	99.9%	12.6	slightly toxic
Rainbow trout	99.7%	1.5	moderately toxic
Coho salmon	99.7%	1.8	moderately toxic
Chum salmon	99.7%	1.8	moderately toxic
Sockeye salmon	99.7%	2.5	moderately toxic
Chinook salmon	99.7%	2.1	moderately toxic
Pink salmon	99.7%	2.7	moderately toxic

Table 20: Acute Toxicity of TCP (3,5,6-TC-2-P) to Freshwater Fish

According to the USEPA RED 1998, a fish early-life stage test was required for triclopyr because the pesticide is intended for use such that its presence in water is likely to be continuous or recurrent regardless of toxicity and there are acute LC_{50} values less than 1 mg/L. The test is begun by placing fertilized eggs in the test chambers and is continued at least until all the control fish are free-feeding. Lethal and sublethal effects are assessed and compared with control values to determine the LOEC and the NOEC. Accordingly a study was submitted and found acceptable for fulfillment of EPA guidelines. The results of the study are provided below:

Species	**% A.I.**	**NOEC & LOEC (ppm)**	**MATC (ppm)**	**Endpoints Affected**
Fathead minnow *(Pimephales promelas)*	44.9	NOEC>104 LOEC<162	130	Length

Table 21: Freshwater Fish Early Life Stage Toxicity-Triclopyr TEA

Wild Mammals, Acute and Chronic

As per the 1998 EPA RED, the results from acute oral rat toxicity studies substitute for wild mammal testing. These toxicity values are reported in the table below (p. 38):

Species	Test Type	Endpoint (Mg/kg/day
Rat	Acute oral LD_{50}	LD_{50}=729 (Males) LD_{50}=630 (Females)
Rat	Two-Generation Reproduction Study Guideline (83-4)	Reproductive/Systemic NOEL = 25 Reproductive/Systemic LEL = 250

Table 22: Wild Mammalian Toxicity - Triclopyr Acid

The above results indicate that triclopyr acid is practically non-toxic to small mammals on an acute oral basis. The 2-Generation rat reproduction study showed that the reproductive/systemic toxicity lowest effect level (LEL) of 250 mg/kg/day was based on decreased litter size, decreased body weight and weight gain, and decreased survival of the F_1 and F_2 litters (EPA RED, p. 38).

Avian Toxicity

The toxic effects of Triclopyr on birds have been investigated in a small number of studies conducted by the Dow Chemical Company. For mallard ducks, acute oral LC_{50} values are reported at 1,698 mg/kg (slightly toxic) for unformulated triclopyr, 3,176 mg/kg for Garlon 3A, and 4,640 mg/kg for Garlon 4. Eight-day subchronic oral LC_{5O} values are reported as follows for the various triclopyr formulations: (Triclopyr Technical Review. MDAR and DEP ADHOC Committee. 1991).

The data summarized below indicate low acute and subchronic toxicity to the bird species tested. No field studies on the toxic effects of Triclopyr or its formulations in birds have been reported

Formulation	Species	Endpoint Value	Toxicity Category
Triclopyr acid	mallard duck	LC_{5O} = 5,000 ppm	Practically non-toxic
	bobwhite quail	LC_{5O} = 2,935 ppm	Practically non-toxic
	Japanese quail	LC_{5O} = 3,278 ppm	Practically non-toxic
Garlon 3A	mallard duck	LC_{5O} = 10,000 ppm	Practically non-toxic
	bobwhite quail	LC_{5O} = 11,622 ppm	Practically non-toxic
Garlon 4	mallard duck	LC_{5O} = l0,000 ppm	Practically non-toxic
	bobwhite quail	LC_{5O} = 9,026 ppm	Practically non-toxic

Table 23: Eight day-subchronic Oral Toxicity for Various Triclopyr Formulations

According to the 1998 EPA RED, reproduction of birds may be affected at levels greater than 100 ppm triclopyr TEA (p.38).

Species	% A.I.	NOEC/LOEC (ppm)	Endpoints Affected
Northern Bobwhite Quail *(Colinus virginicmus)*	98.9	NOEC 500 LOEO500	N/A
Mallard Duck *(Anas platyrhynchus)*	98.9	NOEC 100 LOEC 200	number of 14 day old survivors

Table 24: Avian Reproduction - Triclopyr Acid

Water fowl are likely to be the most highly exposed bird species, given that they swim, drink and feed on lakes and ponds proposed for treatment with Renovate 3; however, based on the following it would appear that there are negligible risks to avian species, including those whose diet might consist primarily of aquatic vegetation treated with triclopyr:

- The toxicity values above indicate that triclopyr is slightly to relatively non-toxic to avian species;
- The nominal EEC in water is ~2.5 mg/L triclopyr as per maximum application rates;
- The property of triclopyr and its metabolites not to accumulate in living tissue; and
- The environmental fate characteristics of triclopyr TEA and triclopyr acid, demonstrating that they are short-lived in the aquatic environment as described graphically in Appendix A, and according to the environmental fate review.

Plant Toxicity

Aquatic Plant - Freshwater Macrophyte, Duckweed *Lemna gibba*

Following EPA's Ecological Effects Test Guidelines, the "Lemna-Test" is the most standardized test using higher plants in bio testing. In a Tier 1 assessment, the effect of triclopyr TEA on two species (five different clones) of duckweed[31] (*Lemna gibba and Lemna minor*) was investigated by Cowgill et.al. using a 7- and 14-day static tests (no renewal of test solutions). Triclopyr concentrations (serial progressive decline of 60%) of 100, 60, 36, 21.6, 13, 7.8, 4.7, and 2.8 mg/L were placed in growth medium with a pH range between 4.6 and 5.4. Results of the effects are reported as the lowest EC_{50}[32] values for the following five endpoints:

Endpoint	Endpoint Value from 7-day Test EC_{50} mg/L	Corresponding Plant Species	Endpoint Value from 14-day Test EC_{50} mg/L	Corresponding Plant Species
Number of plants	40 (7, 72)	*Lemna minor* 7102	24 (7,80)	*Lemna gibba* G-3
Number of fronds (single *Lemna* ''leaf-like'' structure)	35 (68,106)	*Lemna minor* 7102	30 (5, 85)	*Lemna gibba* G-3
Biomass (Dry weight)	48 (16, 80)	*Lemna minor* 7102	26 (12, 61)	*Lemna gibba* G-3
% chlorophyll a	48 (-1, 96)	*Lemna minor* 6591	54 (-13, 120)	*Lemna minor* 6591
% chlorophyll b	53 (9, 97)	*Lemna minor* 6591	99 (31, 168)	*Lemna minor* 7101
Total chlorophyll	49 (3, 95)	*Lemna minor* 6591	58 (-12, 127)	*Lemna minor* 6591

Table 25: Summary of Lowest EC_{50} values for 7- and 14-day tests for the target endpoints (Figures in parentheses denote 95% intervals)

Water hardness, alkalinity and conductivity are reported to have no effect on the toxicity of triclopyr to *Lemna*. Although the authors state that the results from the study indicate that triclopyr is only slightly toxic to duckweed using the "EPA classification scheme", there is no an non-target plant toxicity classification scheme. Therefore, it appears the authors are referring to the EPA toxicity classification scheme for non-target freshwater, estuarine and marine (fish and invertebrates)[33] For purposes of the Department's review, it is important to note that the maximum label application rate result in an EEC's of 2.5 mg/L triclopyr ae and the lowest EC_{50} value was related to the endpoint for reduction in number of plants (*Lemna minor* 7102) at 24 (7,80) mg/L, using the 14-day test. Thus, in conjunction with the rapid dissipation of triclopyr

[31] Duckweeds (*Lemna* spp. are floating fast growing higher plants, spreading from the tropic to the arctic zone. They are primary producers, in that they are a food source for waterfowl, fish, and small animals and serve as physical support for a variety of small invertebrates.

[32] Median effective concentration (EC_{50}): The concentration of material in water to which test organisms are exposed that is estimated to be effective in producing some sublethal response in 50% of the test organisms.

[33] Freshwater fish and invertebrates endpoint mortality LC_{50} values >10 – 100 ppm are classified as "slightly toxic."

residues, label applications of Renovate 3 herbicide appear to pose minimal long-term risks to the native aquatic plant duckweed (Cowgill, 1989).

Following EPA risk assessment methodology, its possible to use the lowest EC_{50} value from this Tier 1 aquatic plant growth test and compare this endpoint with the EEC. When the resulting RQ is greater than an LOC of 1.0, then there is potential for acute risk to aquatic plants. Since the comparison below shows that the RQ = 0.104, it would appear that there is negligible acute risk to duckweed.

Species	**%A.I.**	**Endpoint & Scenario**	**Risk Quotient** EEC / EC_{50}	**LOC – non-endangered spp.**
Duckweed (*Lemna minor* 7102)	32.3% ae	24 mg/L for reduced number of plants -14-day study.	0.104	1.0

Table 26: Risk Quotient for Duckweed Exposed to Triclopry TEA

Aquatic Plant – Freshwater green algae *(Selenastrum capricornutum)* and Freshwater Macrophyte, Duckweed *(Lemna minor*[34]*)*

Environment Canada[35] evaluated the phytotoxicity (24-h inhibition of ^{14}C uptake and 7-day growth inhibition) of the EEC of 23 different pesticides to ten algae spp. and one vascular plant in an effort to examine the question of interspecific sensitivity and its relation to the development of pesticide registration guidelines. Test organisms were selected based on ecological relevance and present use in test protocols. Organisms included green algae (*Scenedesmus quadricauda* and *Selenastrum capricornutum*), diatoms (*Nitzschia* sp. and *Cyclotella meneghiana*), cyanobacteria (*Microcystis aeruginosa, Oscillatoria* sp*., Pseudoanabaena sp., Anabaena inaequalis* and *Aphanizomenon flos-aquae)* and the floating vascular plant, duckweed *(Lemna minor).* Technical or analytical material was used for the testing as per EPA and Environment Canada guidelines. The testing was carried out assuming 10% drift of the maximum label application rate for triclopyr TEA, 3.84 kg/ha (~3.43 lb/A), and an EEC[36] of 2.56 (mg/L). Results from this study indicate that through testing the phytotoxicity of a variety of agricultural pesticides to a wide range of algal taxa, it is evident that there are considerable differences in sensitivity among species and that the use of an uncertainty factor is necessary to provide an acceptable margin of safety in evaluating the hazard presented by these chemicals to the aquatic environment (Peterson, 1994).

Peterson et.al. also provide the data in the table below, which shows that using the maximum EEC, native aquatic macrophyte, duckweed, is the most sensitive aquatic organism tested with an average inhibition of ~ 23%. As represented by the negative percent inhibition, many of the species of cyanobacteria and algae actually show stimulation to growth as compared to controls.

[34] The two primary species tested per the EPA Teir I. Testing Guideline 122-2.
[35] Environment Canada is the federal agency responsible for advising on environmental fate, chemistry and ecotoxicology of pesticides submitted for registration.
[36] Expected Environmental Concentration (EEC): calculated by assuming 10% overspray of maximum application rate to 1-ha of water that is 15 cm deep i.e. 0.384 kg / 1,500,000 L = 2.56 mg/L triclopyr. For details see Appendix B.

Species	Percent inhibition[1]					
	Phenoxyalkanes herbicides		Pyridines herbicides		Brominated herbicides	
	2,4-D	MCPA	Picloram	**Triclopyr**	Bromoxynil	Diquat
	2.917 mg/L	1.400 mg/L	1.760 mg/L	**2.560 mg/L**	0.280 m g/L	0.733 mg/L
Algae						
Cyclotella meneghiana, U2455-D	0 (5)	- 3 (8)	-12 (5)	**-15 (12)**	6 (3)	99* (1)
Nitzschia sp., F110-D	1 (10)	-18* (5)	-7 (21)	**- 4 (3)**	-40* (11)	100* (0)
Scenedesmus Quadricauda, Fll	- 1 (12)	1 (3)	-7 (12)	**13 (9)**	-11 (8)	53* (13)
Selenasirum Capricornutum, U1648	- 2 (9)	-18* (8)	- 2 (8)	**-24* (6)**	14 (2)	69* (8)
Cyanobacteria						
Microcystis, aeruginosa, WPC7820	9 (8)	0 (24)	3 (8)	**-10 (8)**	0 (7)	100* (0)
Microcystis, aeruginosa. U2063	11 (13)	8 (5)	-27 (6)	**-2 (12)**	- 6 (20)	100* (0)
Oscillatoria sp., T129	4 (9)	- 7 (16)	8 (1)	**-9 (3)**	-11 (20)	100* (0)
Pseudoanabaena sp., F63	-7 (6)	19* (2)	15 (10)	**13* (3)**	24 (12)	100* (0)
Anabaena inaequalis, U381	-14 (8)	-15 (11)	14 (8)	**- 4 (13)**	-12 (8)	100* (0)
Aphamzomenon flos-aquae, F107-N	0 (0)	11 (7)	0 (17)	**-34* (16)**	5 (2)	100* (0)
Duckweed						
Leinna minor	34* (5)	42* (3)	10 (5)	**23*(4)**	-4 (2)	100* (0)

[1] Mean **(SD)** % inhibition of ^{14}C uptake for algae and 7-day growth for duckweed, negative values indicate stimulation.

- Statistically different ($P < 0.05$) from controls using two-tailed t-test.

Table 27: Toxicity of Phenoxyacetic Acid, Pyridine and Brominated Herbicides Applied at Expected Environmental Concentrations (EEC) to Cyanobacteria, Algae and Duckweed[37]

Aquatic Plant – Eurasian water milfoil (*Myriophyllum spicatum*. L.)

In an effort to evaluate the selective control of the submersed exotic weed Eurasian water milfoil (*Myriophyllum spicatum*. L.), the U.S. Army Engineer Waterways Experiment Station (USAEWES) treated 6-ha[38] (~14.8 acres) river and 4-ha (~9.9 acres) cove with Garlon 3A at application rates of 2.5 and 1.75 mg/L, respectively, in the Pend Oreille River in the State of Washington, August 1991. Triclopyr was injected 30 – 60 cm below the surface via airboat with 6-stern mounted hoses and attached nozzles fed by a 208-liter tank powered by a pressurized diaphragm pump. Treated water was collected at a depth of 1-m at the river treatment plot and at 0.5 and 0.75 m at the cove treatment plot. Water samples were analyzed for triclopyr residues (detection limit <0.01 mg/L) using a high performance liquid chromatography (HPLC) method

[37] Take from Peterson, HG. et. al. *Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations*. Aquatic Toxicology. Vol. 28, no. 3-4, p. 285, 1994.

[38] 1 hectare (HA) = 2.4710538 acre (A); 1 A = 0.4047 HA.

(percent recovery = 98.12 +/- 0.69). Triclopyr concentrations within the river treatment plot ranged from <0.01 to 0.41 mg/L at 3-days post treatment. Some 675 m downstream the concentrations ranged from <0.01 to 0.47 mg/L, 1-day after treatment. Triclopyr concentrations within the cove treatment ranged from 0.12 to 0.29 mg/L by 7-days after treatment, and ranged from <0.01 to 0.06 mg/L as close as 150 m downstream from the plot. Eurasian water milfoil biomass was reduced by 99% in the treated plot at 4-weeks post-treatment, remained low one-year later (river treatment 28% of pretreatment levels; cove treatment 1% of pretreatment levels). Non-target native plant biomass increased 500-1,000% by one year post-treatment, and remained significantly higher in the cove plot at two-years after treatment. Native species diversity doubled following herbicide treatment (Getsinger, 1997).

Aquatic Plant Toxicity – Coontail, oxygen weed, hydrilla, Brazilian waterweed, and Eurasian water milfoil and Native Submerged Weed Species of Pond Weed, Milfoil spp, and Green Algae spp.

A New Zealand greenhouse study evaluated the effects of triclopyr on the following exotic submerged target weed species **coontail** (*Ceratophyllum demersum* L.); **oxygen weed [**Lagarosiphon (*Lagarosiphon major* (Ridley) Wager)]; **hydrilla** (*Hydrilla verticillata* (LF) Royle); and **Brazilian waterweed [egeria** (*Egeria densa* Planch)] and on the following non-target native submerged weed species of **pond weed spp.** [*Potamoggton ochireoius* Raoul, *Potamogeton cheesemanii* A. Benn]; **milfoil spp.** *[Myriophyllum triphyllum* Orchard, *Myriophyllum propinquun* A. Cunn]; **green algae spp.** [*Chara carallina* Willd, *Chara globularis* Thuill, *Nitella hookeri* A. Br, *Nitella leptostachys* A. Br, and *Nitella pseudo-flabellata* A. Br.]. The experiments took place in a greenhouse using four 170 L tanks where at least 15-plants/spp. were placed in each treatment tank prior to herbicide application. Triclopyr (Garlon 3A) was added at concentrations of 0, 0.25, 1 and 2.5 mg/L triclopyr to the four tanks. The results of this study show only transient growth effects in the target plants treated with triclopyr. These target weeds varied in their level of susceptibility with oxygen weed the most susceptible and exposure for 11-days at high rates achieved less than 50% plant kill. Triclopyr produced epinastic shoots in target species, except the charophytes[39]**.** Similarly the native macrophytes[40] such as the milfoil and pond weeds had epinastic shoots, which were particularly apparent in the milfoils, with some loss of turgid; however, the milfoil species were not controlled by triclopyr (Hofstra, 2001).

Aquatic Plant Toxicity – Targets Plants Purple Loosestrife

In 1991 triclopyr TEA was applied at 4.0, 8.0, and 12.0 kg a.i./ha or ~ 3.56, 7.13, and 10.87 lbs. a.i./a. for the removal purple loosestrife (*Lythrum salicaria*). The goal of the study was observe the response of native vegetation and subsequent impact on loosestrife germination in a southern Ontario wetland during 1993 and 1994. Different levels of adult loosestrife control were achieved. The lowest density of adult loosestrife was observed in the 8.0 and 12.0 kg a.i./ha treatment plots where root kill was most effective. Most new adult loosestrife plants in the 8.0 and 12.0 kg a.i./ha treatment plots established from seed. The highest densities of loosestrife seedlings and grass (Grammineae) species were observed in the treatment plots with the lowest number of adult loosestrife plants (12.0 kg a.i./ha). Sedge species (*Carex spp.*) did not differ between treatment levels during 1993 and 1994. Loosestrife seedling densities decreased from 1993 to 1994, suggesting that increased native plant species might slow the rate of loosestrife

[39] Charophytes such as Spirogyra, stoneworts, and desmids are all members of the fresh-water group of "green algae" that are single-celled to complex multicellular organisms. They are important constituents in the food chain, but some species can cause blooms in eutrophic lakes.

[40] Aquatic macrophytes are large aquatic plants that are visible to the naked eye; in other words, they are larger than most algae. The general term "aquatic plants" usually refers to aquatic macrophytes, but some scientists use it to mean both aquatic macrophytes and algae.

reestablishment from the seed bank. The native vegetation appears to replace adult loosestrife for a limited time following herbicide application, however, adult loosestrife plants produce a great quantity of seeds per year and without subsequent treatment, loosestrife will slowly reinvade a wetland. (Gabor, 1996).

Aquatic Plant Toxicity – Eurasian watermilfoil and Monocots Elodea, Sago Pondweed, and Vallisneria

A study was conducted to use the activation of increased synthesis of the oxidative enzyme[41] peroxidase (PRX) as a characteristic physiological responses to treatment of Garlon 3A, triclopyr TEA, in four plant species at 1 mg ae/L for 12-hr, and 2.5 mg ae/L for 24- hr. Guaiacol-specific PRX levels increased rapidly within 1.5 days after triclopyr application in the dicot Eurasian watermilfoil (*Myriophyllum spicatum L.*). The non-target monocots elodea (*Elodea canadensis Rich.*), sago pondweed [42](*Potamogeton pecti-natus* L.), and vallisneria (*Vallisneria americana* Michx.) showed no visual effects of triclopyr treatment through 8-DAT, and PRX levels were unchanged in treated and untreated plants during this time. Only milfoil exhibited the epinastic curvature of apical and axillary shoots characteristic of auxin-like compounds. Symptoms occurred by 3-DAT and epidermal rupture was evident from presence of extracellular gas bubbles in stems. Treated plants became water-logged and began to decompose, and by 14-DAT no viable stems or leaves remained. No regrowth occurred in either treatment level, and no tissue remained for biomass harvest at 37-DAT. Biomass of sago pondweed was reduced by 60% 35-DAT at the 2.5 mg/L triclopyr rate (the maximum label rate). The authors suggest that the early PRX response to triclopyr effect, which differentiated Eurasian watermilfoil from non-target species, may be predictive of rapid susceptibility to this herbicide and may support the use of triclopyr to remove the exotic dicot milfoil while maintaining the native monocots elodea and vallisneria. These results are also consistent with field studies concducted in Washington State by Getsinger et al. and included in this review (Sprecher, 1995).

Aquatic Plant Toxicity – Eurasian watermilfoil native submersed species, sago pondweed (*Potamogeton pectinatus* L.)

In another study three aquatic herbicides effective on the exotic weed Eurasian watermilfoil (*Mvriofihyllum spicatum* L.), endothall, 2,4-D, and triclopyr TEA, were evaluated in the laboratory for selective control of the native submersed species, sago pondweed (*Potamogeton pectinatus* L.). For each herbicide, three concentrations in ranges associated with Eurasian watermilfoil or sago pondweed control were applied in static exposures of 24-hr, and plants were monitored for 35-d. Some 4-DAT there were marked differences among treatments with plant canopies in aquaria treated with endothall already showing a brownish appearance; those treated with triclopyr and 2,4-D remained bright green. Most damage was seen at the highest endothall rate, 2 mg/L for 24 hr. Endothall at 0.5, 1, and 2 mg/L significantly reduced final biomass by > 72%, confirming that this herbicide will not maintain populations of sago pondweed where it is used to manage Eurasian watermilfoil. Application of the systemic herbicides, 2,4-D and triclopyr at 1, 1.5 and 2 mg/L resulted in no significant reduction in biomass from 2,4-D, but up to 24% reduction with triclopyr. The lowest triclopyr concentration did not reduce pondweed biomass significantly. Although treatment with 1.5 or 2 mg/L triclopyr significantly decreased biomass production by > 22%, plants maintained full canopies and underwent normal life-cycles.

[41] Increased synthesis of the oxidative enzymes often occurs in response to various biotic and abiotic stresses in plants. Other oxidative enzymes include superoxide dismutase, catalase, glutathione reductase. and polvphenol oxidase.

[42] Sago pondweed is a submersed perennial macrophyte, native to a range of fresh, alkaline, and brackish waters in marshes,lakes,and streams of the United States. The submersed morphology of sago pondweed subjects it to displacement by thick surface canopies produced by non-native weed species such as Eurasian watermil-foil (Sprecher, 1998).

Exposures of 24-hr at these concentrations effectively control milfoil, eliminating 85% of biomass. However, the author points out that earlier research shows that an exposure of 24-hr to 2.5 mg/L triclopyr reduced sago pondweed biomass by two-thirds. Thus concentration exposure times (CETs) of 1.5 to 2 mg/L triclopyr for 24 hr are indicated for targeting millfoil where subsequent rapid recovery of sago pondweed populations from plants is desired. Results from both 2,4-D and triclopyr indicate that they are able to eliminate or greatly reduce the presence of milfoil in the field at rates that allow for rapid recovery and recolonization by sago pondweed. The authors suggest that with treatment early in the year, milfoil is expected to be readily controlled at lower rates with subsequent regrowth of the more resistant sago pondweed from tubers and rhizomes as well as plants (Sprecher, 1998)..

Relative to EPA guidelines, Dow (primary registrant) has fulfilled all requirements for non-target aquatic plant toxicity testing with triclopyr TEA. The results indicate that exposure to triclopyr TEA at levels of 8.80 ppm or greater may cause detrimental effects to the growth and reproduction of vascular aquatic plant species. Algae or diatoms may be affected from exposure levels of greater than 5.9 ppm ai triclopyr TEA or 32.45 ppm ai of tric1opyr acid (1998 RED, p.49.)

Species	**% A.I.**	**EC_{50} (ppm ae)**	**EC_5 or NOEC (ppm ae)**
Marine diatom *Skeletonema costatum*	45.01%	6.70	0.40
Duckweed *(Lemna gibba)*	45.01%	8.80	3.5
Duckweed *Lemna gibba*	45.00%	11.00	3.5
Blue-green algae *Anabaena flos-aquae*	45.0%	5.90	2.0
Freshwater microalga *Kirchneria subcapitata (Selenastrum Capricornutum)*	45.01%	7.60	11.3
Diatom *Navicula pelliculosa*	45.0%	15.30	8.0
Freshwater microalga *Selenastrum capricornutum*	98.8% triclopyr acid	32.5	7.0

Table 28: EC_{50} and EC_5 Values for Various Aquatic Plants and Phytoplankton Exposed to Triclopyr TEA

References:

Cessna, Allan J. 2002Environmental Fate of Triclopyr. Reviews of Environmental Contamination and Toxicology. Vol. 174; pp.19-48.

Cowgill, UM; Milazzo, DP; Landenberger, BD. 1989. A comparison of the effect of triclopyr triethylamine salt on two species of duckweed (Lemna) examined for a 7-and 14-day test period. Water Research. vol. 23, no. 5, pp. 617-623.

Edginton, Andrea N.; Stephenson, Geraldson R.; Sheridan, Patrick M.; Thompson, Dean G.; and Boermans, Herman J. 2003. Effect of pH and Release® on Two Life Stages of Four Anuran Amphibians. Environmental Toxicology and Chemistry, Vol. 22, No. 11, pp. 2673-2678.

Foster, D.R.; Getsinger KD; Petty, D G. 1997. The Aquatic Dissipation of Triclopyr in a Whole Pond Treatment. Study ID: ENV95012.

Gabor, TS; Haagsma, T; Murkin, HR. 1996. Wetland plant responses to varying degrees of purple loosestrife removal in southeastern Ontario, Canada. Wetlands. No. 1, pp. 95-98.

Gersich, FM; Mendoza, CG; Hopkins, DL; Bodner, KM. 1984. Acute and chronic toxicity of triclopyr triethylamine salt to Daphnia magna Straus. Bulletin of Environmental Contamination and Toxicology. vol. 32, no. 4, pp. 497-502,.

Getsinger, KD; Turner, EG; Madsen, JD; Netherland, MD. 1997. Restoring native vegetation in a Eurasian water milfoil-dominated plant community using the herbicide triclopyr. Regulated Rivers: Research & Management. Vol. 13, no. 4, pp. 357-375..

Goodman, D.G. and Hildebrandt, P.K. 1996 Review of the Tumor Data from the Chronic Toxicity/Carcinogenicity Studies of Triclopyr in F344 Rats and ICR (Jcl:ICR) Mice. Pathco, Inc. .

Hamaker, J.W.1975. Adsorption of Triclopyr in Soil. Dow Chemical USA

Hofstra, DE; Clayton, JS. 2001. Evaluation of Selected Herbicides for the Control of Exotic Submerged Weeds in New Zealand: I. The Use of Endothall, Triclopyr and Dichlobenil. Journal of Aquatic Plant Management. Vol. 39, pp. 20-24.

Janz, David M.; Farrell, Anthony P.; Morgan, J.D.; and Vigers, Gary A. Acute Physiological Stress Responses of Juvenile Coho Salmon (*Oncorhynchus kisutch*) to Sublethal Concentrations of Garlon 4®, Garlon 3A® and Vision® Herbicides. Environmental Toxicology and Chemistry. Vol. 10, pp. 81-90.

Knuteson, J.A.. 1999. Review of the Environmental Fate of 3,5,6-Trichloro-2-pyridonol (TCP): Laboratory, Terrestrial, and Aquatic Field Studies. Dow AgroSciences LLC. Study ID:GH-C 4875.

Kreutzweiser, DP; Holmes, SB; Eichenberg, DC. 1994. Influence of exposure duration on the toxicity of triclopyr ester to fish and aquatic insects. Archives of Environmental Contamination and Toxicology. Vol. 26, no. 1, pp. 124-129.

Lickly, TD; Murphy, PG. 1987. The amount and identity of (super(14)C) residues in bluegills (Lepomis macrochirus) exposed to (super(14)C) triclopyr. Environment International. vol. 13, no. 2, pp. 213-218.

Maloney, RF. 1995. Effect of the herbicide triclopyr on the abundance and species composition of benthic aquatic macroinvertebrates in the Ahuriri River, New Zealand. New Zealand Journal of Marine and Freshwater Research. Vol. 29, no. 4, pp. 505-515.

McCall, P.J. and P.D. Gavitt. 1986. Aqueous Photolysis of Triclopyr and its Butoxyethyl Ester and Calculated Environmental Photodecomposition Rates. Environmental Toxicology and Chemistry. Vol 5. pp 879-885.

Perkins, Peggy J.; Boermans, J. Herman; and Stephenson, R. Gerald. Toxicity of Glyphosate and Triclopyr Using the Frog Embryo Teratogenesis Assay-Xenopus. Environmental Toxicology and Chemistry, Vol. 19, No. 4, pp. 940–945, 2000.

Perschbacher, PW; Ludwig, GM; Slaton, N. 2002. Effects of common aerially applied rice herbicides on the plankton ommunities of aquaculture ponds. Aquaculture. Vol. 214, no. 1-4, pp. 241-246. 15..

Peterson, HG; Boutin, C; Martin, PA; Freemark, KE; Ruecker, NJ; Moody, MJ. 1994. Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations. Aquatic Toxicology. Vol. 28, no. 3-4, pp. 275-292,

Ritter, Amy, Alan Peacock. 2000. Aquatic Dissipation Modeling of Triclopyr. Dow AgroSciences. Study WEI 396.06.

Solomon, Keith R., Cindy S. Bowgey, Karsten Liber, Gerald Stephenson. 1988. Persistence of Hexazinone (Velpar), Triclopyr (Garlon), and 2,4-D in a Northern Ontario Aquatic Environment. J Agric Food Chemistry. 36, 1314-1318.

Sprecher, SL; Stewart, AB. 1995. Triclopyr effects on peroxidase activity in target and non-target aquatic plants. Journal of Aquatic Plant Management. Vol. 33, pp. 43-48,.

Sprecher, SL; Getsinger, KD; Stewart, AB. 1998. Selective Effects of Aquatic Herbicides on Sago Pondweed. Journal of Aquatic Plant Management. Vol. 36, pp. 64-68.

USEPA.
(i) 1998. Registration Eligibility Document. Triclopyr. EPA 738-R-98-011. http://www.epa.gov/oppsrrd1/REDs/2710red.pdf

(ii) 1998. RED Factsheet. Triclopyr EPA738-F-98-007. pp5 http://www.epa.gov/oppsrrd1/REDs/factsheets/2710fact.pdf

Vencill, William K. 2002 WSSA Herbicide Handbook. 8th Edition

Woodburn, K.B.1988. The Aquatic Dissipation of Triclopyr in Lake Seminole, Georgia. Laboratory Project ID GH-C 2093. Dow Elanco.

Woodburn, Kent, Fred R. Batzer, Frank H White, and Mark R Schultz. 1993.The Aqueous Photolysis of Triclopyr. Environmental Toxicology and Chemistry. Vol 12. pp 43-55. USA Pergamon Press Ltd.

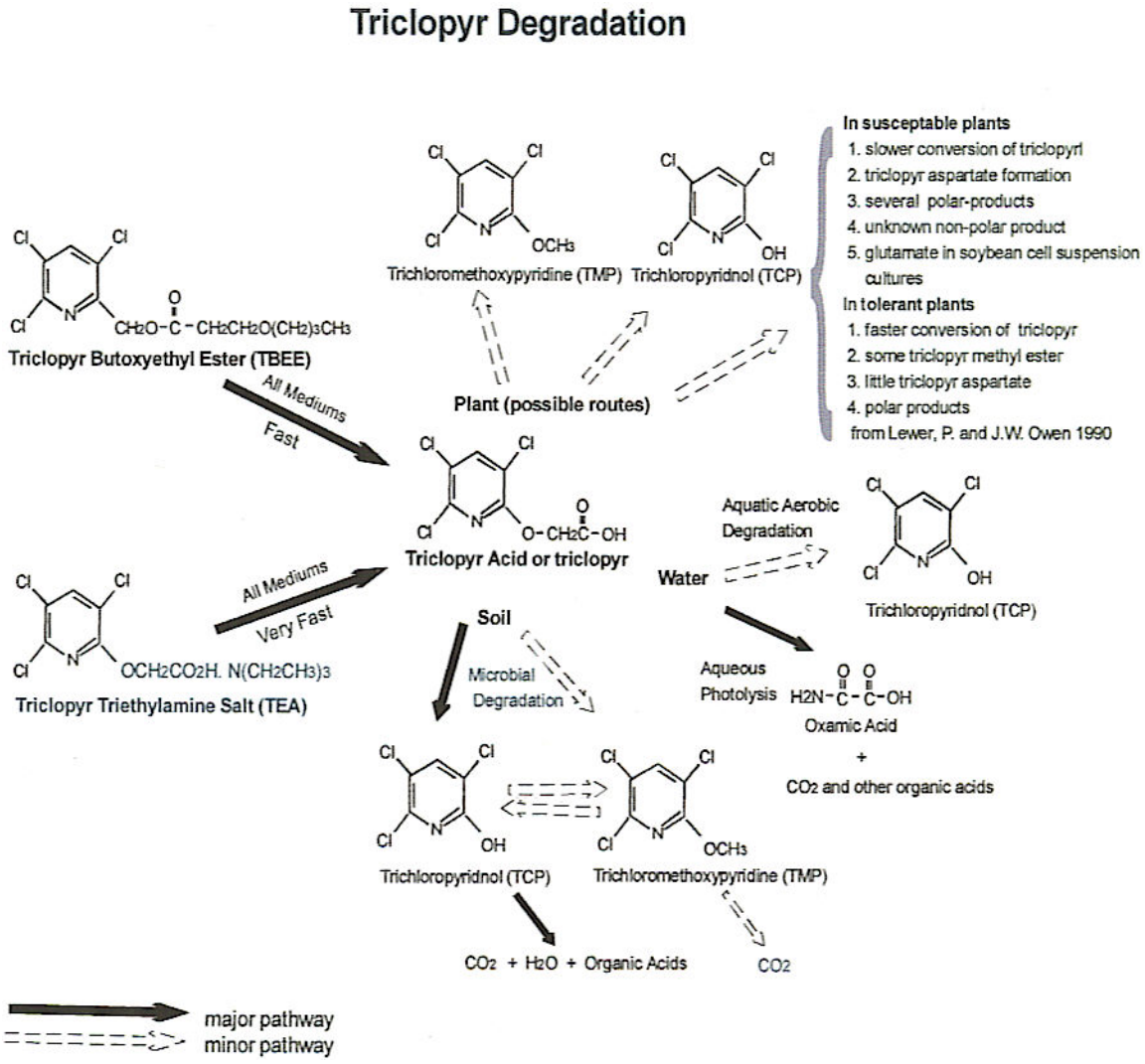
Wolt, J.D., 1995, Anaerobic Aquatic Metabolism of 14Ctriethylamine". Unpublished study conducted by North American Environmental Chemistry Laboratory, Indiana. Dow Study Number 43837502

Yucheng, Feng; Minard, Robert D.; and Bollag, Jean-Marc. 1998. Environmental Toxicology and Chemistry. Vol 17 No 5. pp 814-819.

Appendix A: Triclopyr Degradation Pathway

Taken from the ENVIRONMENTAL FATE OF TRICLOPYR by Carissa Ganapathy, Environmental Monitoring & Pest Management Branch, Department of Pesticide Regulation, Sacramento, CA 95814-5624. (January 2, 1997).

http://www.cdpr.ca.gov/docs/empm/pubs/fatememo/triclopyr.pdf



Appendix B Calculating the Nominal EEC for the Peterson, 1994 Study

Confirmation of the EEC based on label application of 3.84 kg triclopyr/HA to a 1 HA pond 15 cm deep and assuming 10% drift and uniform mixing.

1 HA = 100m x 100m = 10,000 m^2

100 m x 100 m x 0.15 m = 1,500 m^3

1 m^3 H_2O = 1,000 L H_2O

1,500 m^3 x 1,000 L H_2O / 1 m^3 = 1,500,000 L H_2O / HA

Application Rate = 3.84 kg triclopyr / HA

Drift = 10% of 3.84 kg / HA or 0.384 kg/HA

EEC = 3,840,000 mg/1,500,000 L H_2O

EEC = 2.56 mg/L

Appendix C: Calculating the Nominal EEC Based on the Label Application Rate

Confirmation of the maximum EEC based on label application rate to 1 Acre of water that is 1-ft. deep and assuming uniform mixing.

Rate of application is 2.3 gallons Renovate 3 per 1 acre of water that is 1-ft deep.

1 ft^3 water = 7.48 gallons water
1 acre water at 1-ft depth = ~208.71 ft x ~208.71 ft x 1ft = ~ 43,560 ft^3 water
43,560 ft^3 x 7.48 gallons water/1 ft^3 = 325,828.8 gal. water
325,828.8 gal. x 3.785 liters/1 gal. = 1,233,262 Liters water
3 lb. Triclopyr ae/Gallon x 2.3 gallons Renovate = 6.9 lb. Triclopyr ae
6.9 lb. Triclopyr ae x 454 g/1 lb. = 3,132.6 g triclopyr ae = 3,132,600 mg triclopyr ae
3,132,600 mg triclopyr ae/1,233,262 Liters water = ~2.5 mg/L or ~2.5 ppm

Rate of application is 2 quarts Renovate 3 per 1 acre of water that is 1-ft deep.

1 ft^3 water = 7.48 gallons water
1 acre water at 1-ft depth = ~208.71 ft x ~208.71 ft x 1ft = ~ 43,560 ft^3 water
43,560 ft^3 x 7.48 gallons water/1 ft^3 = 325,828.8 gal. Water
325,828.8 gal. x 3.785 liters/1 gal. = 1,233,262 Liters water
3 lb. Triclopyr ae/Gallon x 0.5gallons Renovate = 1.5 lb. Triclopyr ae
1.5 lb. Triclopyr ae x 454 g/1 lb. = 681 g triclopyr ae = 681,000 mg triclopyr ae
681,000 mg triclopyr ae/1,233,262 Liters water = ~0.55 mg/L or ~0.55 ppm

Appendix D: Alternative Control Materials[43]

Sample Product Names†	EPA Reg. No.	Active Ingredient	Use	Notes
Reward Landscape and Aquatic Herbicide	100-1091 10182-404	Diquat dibromide	Non-selective, contact type, herbicide	For control of many submerged and floating aquatic macrophytes and some types of filamentous algae in static and low-turbidity water. When used as an aquatic herbicide at recommended application rates, diquat residues in water decrease rapidly to essentially undetectable levels within 7-14 days (Labeling and 2004 GEIR, Appendix III).
AquaPro Aquatic Herbicide	62719-324-67690	Glyphosate	Non-selective, foliar absorbed systemic herbicide	For control of annual and perennial weeds and woody plants in and around aquatic and other noncrop-sites; e.g. brush, cattail and other emergent plant problems (Labeling.
Sonar A.S.	67690-4	Fluridone	Selective systemic herbicide	Primarily use for control of broad-leaved, submerged aquatic macrophyte species including Eurasian watermilfoil, curly-leaf pondweed as well as native pondweeds in freshwater ponds, lakes, reservoirs, potable water sources, drainage canals and irrigation canals (Labeling and 2004 GEIR, Appendix III).
Captain aquatic algaecide	67690-9	Copper Carbonate	Algicide	Primarily used for control of planktonic and filamentous algae and certain vascular plants in potable water sources, lakes, rivers, reservoirs, and ponds, slow-flowing or quiescent water bodies, crop and non-crop irrigation systems (canals,laterals, and ditches), fish ponds, golf courses, ornamental, swimming, and fire ponds, and fish hatcheries (Labeling. 2004 GEIR, Appendix III).
Aquathol Super K	4581-388	Endothall	Contact herbicide	Primarily used for the control of submersed weeds (2004 GEIR, Appendix III).
Aqua-Kleen	228-378-4581	2,4-D	Somewhat selective systemic herbicide	Primarily used for the control of submersed, emersed and floating aquatic broadleaf plants. MDEP has a policy of discouraging the use of 2,4-D in lakes that constitute a water supply or may substantially contribute to groundwater that might serve as a drinking watersource (2004 GEIR, Appendix III).
Aquashade Aquatic dyes	33068-1	mixture of blue and yellow dyes (Erioglaucine and Tartrazine)	Nonselective herbicide/algaecide	Primarily used for the control young, bottom-growth of plants in contained lakes and ponds. Aquashade filters out the red-orange and blue-violet wavelengths of light from the sunlight spectrum, thus interfering with the photosynthetic process in plants (2004 GEIR, Appendix III).

† Products listed above were randomly chosen to provide examples of alternative aquatic hebicides registered for use in Massachusetts and are not provided as a recommendation from the Department.

[43] The Department maintains the following website relative to aquatic weed management with links to those products registered for use in Massachusetts:
http://www.mass.gov/agr/pesticides/water/Aquatic/profile.htm
Additional product and chemical information is provided in the 2004 GEIR for Eutrophication and Aquatic Plant Management in Massachusetts, Chapter 2.0 Case Histories of Lake Management in Massachusetts and Chapter 4.0 Methods to Control Aquatic Plants, which is maintained on the following Department of Conservation and Recreation, Lakes and Ponds Program website:
http://www.mass.gov/dem/programs/lakepond/lakepond.htm