Healthy Forest Partnership "Early Intervention Strategy" report on water monitoring within the 2018 research areas for Foray® 76B *Bacillus thuringiensis* var. kurstaki (*Btk*) and Limit® 240LV (tebufenozide)

Emily Owens[1] and Dr. Rob Johns[1]

BACKGROUND

A water monitoring project was initiated by the Healthy Forest Partnership's "Early Intervention Strategy" in 2015 to monitor and educate the general public on the use of *Btk* and tebufenozide during the ongoing spruce budworm research projects. The following is the report from the 2018 water monitoring program.

Spruce Budworm

Spruce budworm (*Choristoneura fumiferana*) is a native insect in North America that outbreaks every ~35 years, during which it causes serious defoliation that may result in tree mortality to spruce and fir trees. Over the past several years, an outbreak in Québec has experienced multiple years of defoliation. South of Québec, the outbreak has begun to spread into northern New Brunswick and work is underway to develop a strategy to limit its spread and impact.

Btk

One of the main treatment products proposed for use is *Bacillus thuringiensis* var. *kurstaki* (*Btk*), a naturally occurring bacterium favored for use in agriculture and gardening due to its effectiveness in managing larval pests while having no effect on humans, other mammals, birds, fish, or amphibians (Meher et al. 2002.) *Btk* is also useful for managing spruce budworm and is being used in the Québec outbreak by the Société de protection des forêts contre les insectes et maladies (SOPFIM). *Btk* is deposited on the needles of the spruce and fir trees and is eaten by the budworm larvae. In brief, *Btk* is only toxic to specific groups of insects (e.g., feeding caterpillars), its toxicity is caused by ingestion of protein crystals produced by *Bt* spores and "broken down" by the strong alkalinity of the insect's gut. The enzymes released through this process are what injures or kills the insect, through its action on the gut lining (Henry, 2014). Note that the guts of vertebrates are highly acidic and thus when ingested the *Btk* crystals simply pass harmlessly through the digestive system. After decades of use and testing, there is not a single instance of demonstrated toxicity to fish, mammals, birds, amphibians, or any aquatic organism. Only feeding larvae are susceptible (Natural Resources Canada, 2016).

Tebufenozide

Another one of the main treatment products proposed for use is Limit® 240LV (tebufenozide), an insect growth regulator that, when ingested by the spruce budworm larvae, interferes with its molting ("shedding its skin") causing the spruce budworm to stop feeding and die (Natural Resources Canada, 2016). Tebufenozide is deposited on the needles of the spruce and fir trees and is eaten by the budworm larvae. The larvae stop feeding almost immediately and die within a day or two. Tebufenozide has no adverse effects on birds, mammals, aquatic species, or soil invertebrates (US Department of Agriculture, 2012; Sundaram, 1997). Only feeding larvae are susceptible (Natural Resources Canada, 2016).

[1]Natural Resources Canada, Canadian Forest Service - Atlantic Forestry Centre, Fredericton, New Brunswick, Canada, E3B 5P7

SAMPLING PROTOCOL

Water samples were collected from the Campbellton's Walker Brook Comp watershed (Prichard Lake Intake), the Eel River Watershed (Eel River and its intake site), the Charlo River watershed (Charlo River and its intake site) (Fig.1), the Petit Rocher Watershed (Nigadoo River and its intake site), and Bathurst Harbour watershed (Middle River and its intake site) (Fig. 2). Water samples were also collected from the Miramichi River (Miramichi West and East) (Fig. 3). The Campbellton, Eel River, Petit Rocher, Dalhousie, and Bathurst watersheds were treated within or nearby the *Btk* treatment area and two sites in the Miramichi River were treated within or nearby the tebufenozide treatment area. Samples were collected at the intervals: 1) within three days prior to initial treatment of *Btk*, 2) within three days following final treatment, and 3) three weeks following final treatment for *Btk* and two weeks for tebufenozide. These sampling periods generally follow the procedures developed and used by SOPFIM for *Btk* for the past two decades and we chose to follow this protocol for tebufenozide as well to mimic recent methodology in tebufenozide laboratory testing (Kreutzweiser and Nicholson, 2007). Due to a timing error, no sample prior to treatment for the Petit Rocher watershed was collected. Water samples from all sampling sites were collected along the shore (4 samples at approximately 10 meter intervals).

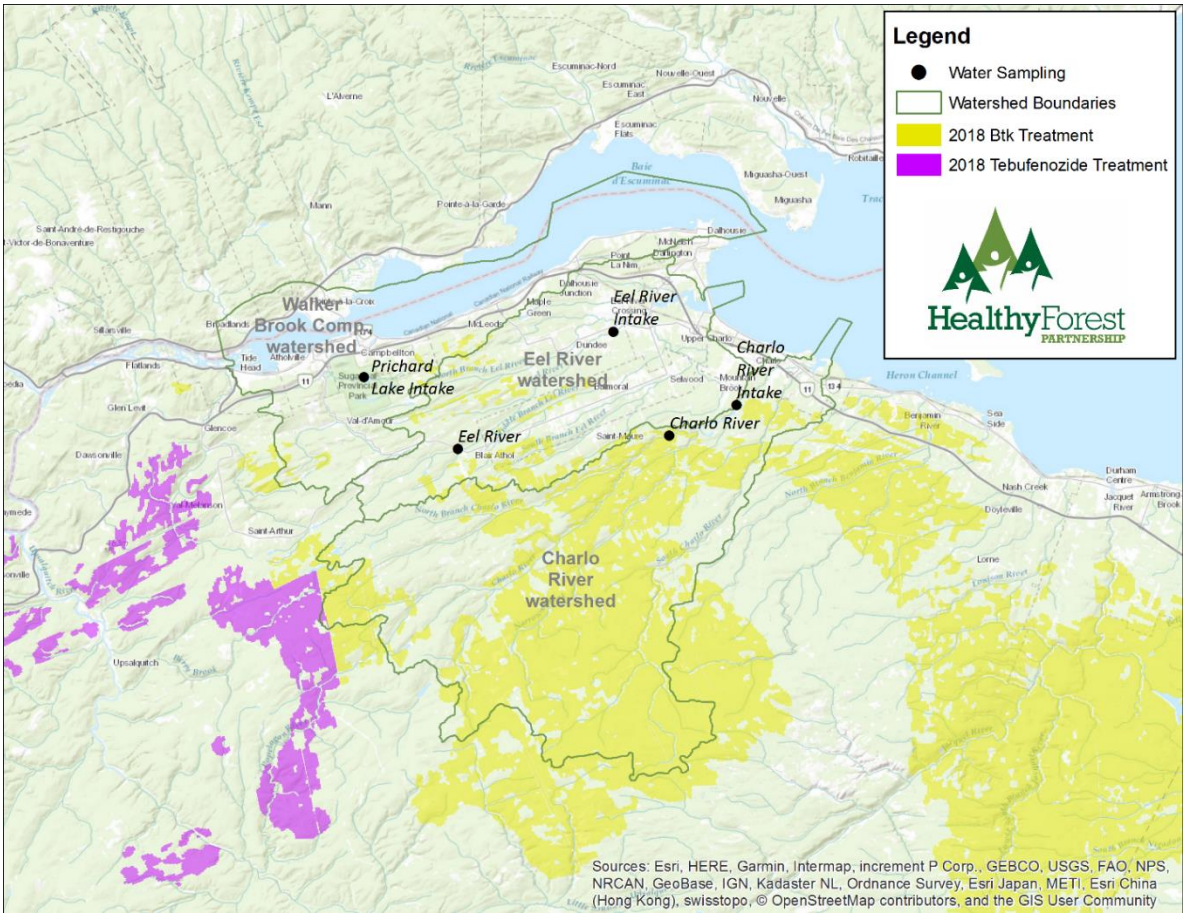

Figure 1. Map of the Campbellton's Walker Brook Comp watershed, Eel river watershed and Charlo River watershed's water sampling sites and areas treated.

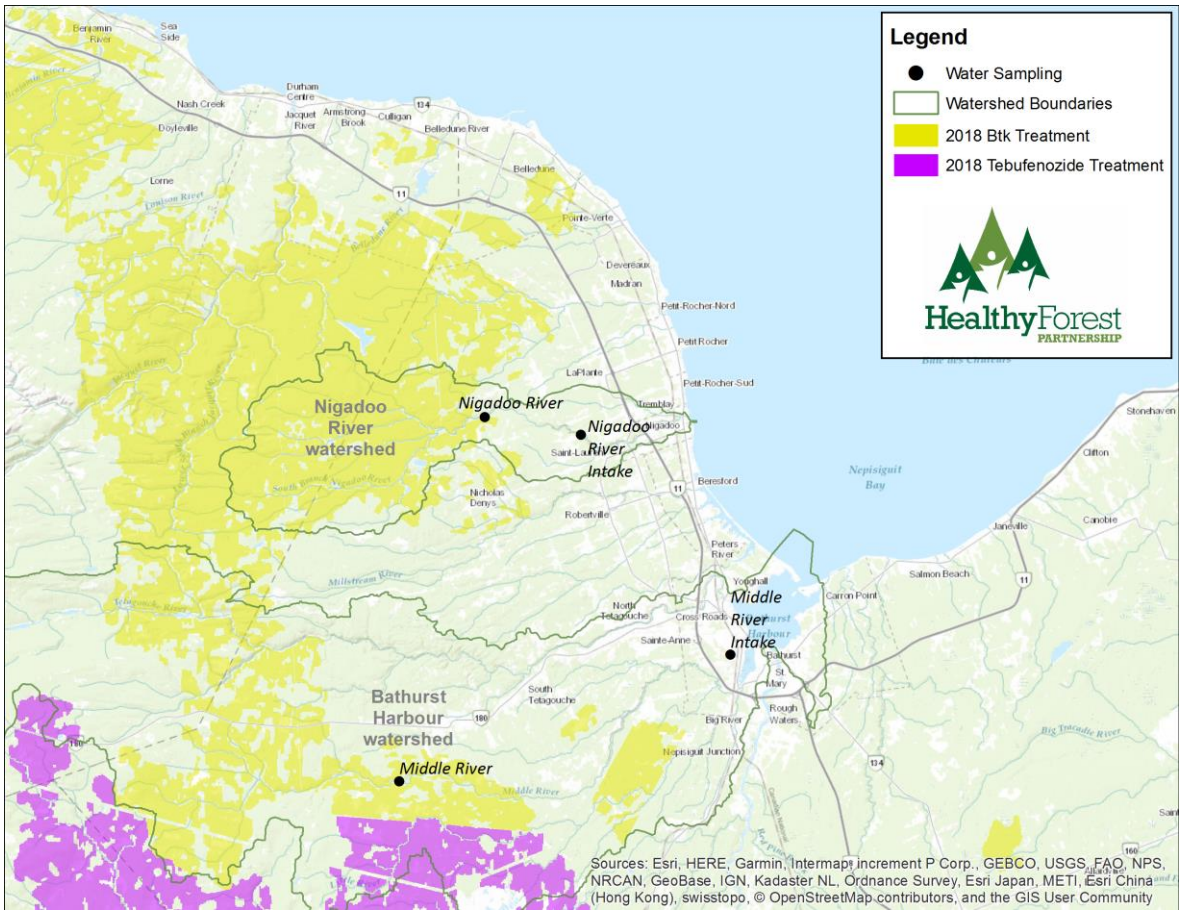

Figure 2. Map of the Nigadoo River watershed and Bathurst Harbour watershed's water sampling sites and areas treated.

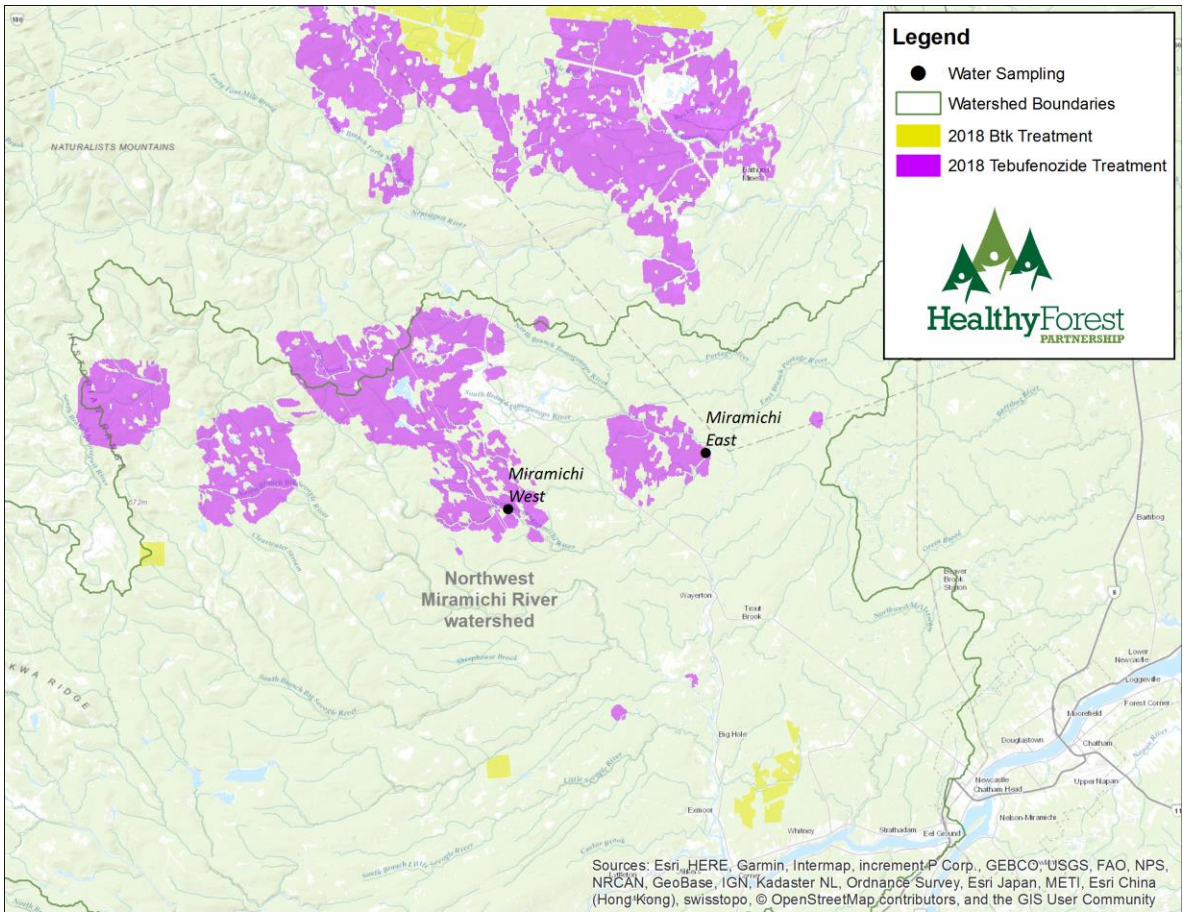

Figure 3. Map of the Northwest Miramichi River watershed's water sampling sites and areas treated.

RESULTS AND DISCUSSION

Btk

Results from laboratory analyses done by RPC in Fredericton, show that concentrations of *Btk* increase from a very low initial pre-treatment level to a maximum mean concentration of 0.11 CFU/ml in the Campbellton Watershed at the Prichard Lake intake site. In the Eel River Watershed, a mean concentration of 2.2 CFU/ml in the Eel River and 0.84 CFU/ml at its intake site. In the Petit Rocher, a mean concentration of 11.6 CFU/ml in the Nigadoo River and 5.7 CFU/ml at its intake site. In the Dalhousie watershed, a mean concentration of 2.0 CFU/ml in Charlo River and 2.8 CFU/ml at its intake site. In the Bathurst Watershed, a mean concentration of 4.6 CFU/ml in the Middle River and 7.4 CFU/ml at its intake site (Appendix 1).

Several studies have been conducted on the safety of *Bt* variants for non-target organisms following environmental exposure, especially for aquatic and soil-dwelling organisms (Beavers and Smith, 1990; Christensen, 1990; EPA, 1998). For rodents, no observable effects (e.g. changes to behavior, body mass or organ condition) were found for concentrations of 125 billion CFU/mL (Meher et al. 2002), over 200,000 times the highest mean concentration detected from the rivers sampled from this water monitoring project. Other non-target insect groups were reported to show no toxicity at concentrations over 100,000 CFU/mL (EPA, 1998). The general lack of *Btk* toxicity for mammals has been reported often over the last 30 years, which has led the World Health Organization (WHO) to conclude that the use of *Btk* is safe for agricultural, horticultural and silvicultural control of pest insects (WHO, 1999). The WHO further notes that *Btk* is unlikely to pose a hazard to humans and other vertebrates because of the mechanisms underlying toxicity of *Btk* to target insect species (WHO, 1999) and the Environmental Protection Agency (EPA) waived requirements for future toxicity studies for *Btk* in acknowledgement of its lack of health hazards to humans.

Tebufenozide

Results from laboratory analyses of the Miramichi water samples done by RPC in Fredericton, NB show trace levels of tebufenozide with a mean maximum concentration of 0.000008 mg/L two days following treatment and 0.0000 15mg/L two weeks following treatments in the Miramichi West site. In the Miramici East site, trace levels of tebufenozide with a mean maximum concentration of 0.0003 mg/L two days following treatment was found and no tebufenozide was found in the two week following treatment sample (Appendix 2).

Several studies have been conducted on the safety of tebufenozide and at the worst case expected, there were no significant effects on exposed test species (Kreutsweiser and Capell, 1994). Even at the maximum tested concentration of 3.5 mg/L or 100x the expected environmental concentrations, there were no significant effects on survival of the test species including soil dwelling invertebrates and macro-invertebrates (Kreutsweiser and Capell, 1994; Addison, 1996). Research shows that 90 to 95% of tebufenozide is deposited in the forest canopy, is relatively rainfast and is not easily washed off by rainfall (Kreutzweiser & Nicholson, 2007; Sundaram, 1995). Tebufenozdie that lands on water has no significant harmful effects on most organisms including aquatic invertebrates (Kreutzweiser & Nicholson, 2007. Kreutzweiser

et al. 1994; 1998). The portion that reaches the ground stays in the upper 5 cm of the ground, is broken down over time by soil microbes, sunlight, and moisture and is not harmful to soil dwelling invertebrates (Sundaram, 1997; Thompson & Kreutzweiser, 2007; Addison, 1996). Even at the highest levels of tebufenozide ever tested for mammals and birds, no toxicity was found (USDA Forest Service Risk Assessment document).

CONCLUSIONS

Based on the results from this report, the 2018 Early Intervention Strategy research treatments resulted in what are essentially trace levels of *Btk* and tebufenozide within and nearby watersheds. Even at worse case scenarios and the highest levels tested, no adverse effects was found on birds, mammals or aquatic species for both *Btk* and tebufenozide. Both products were studied extensively by scientists and regulatory officials before being allowed for use in Canada (Natural Resources Canada 2016).

APPENDIX 1: Mean (± standard error) concentrations of *Btk* reported from water samples collected from the 2018 Early Intervention Strategy research area, reported as colony forming units per milliliter (CFU/mL).

Watershed	Site Name	Latitude	Longitude	Pre-treatment	+3days	+3weeks
Campbellton	Prichard Lake Intake	47.98142	-66.66550	0.063 ± 0.023	0.11 ± 0.042	0.003 ± 0.003
Eel River	Eel River	47.94029	-66.58497	0.085 ± 0.028	2.2 ± 0.208	0.153 ± 0.031
Eel River	Eel River Intake	48.00761	-66.45190	0.838 ± 0.804	0.84 ± 0.187	0.038 ± 0.009
Petit Rocher	Nigadoo River	47.75233	-65.86410	n/a	11.575 ± 6.813	1.950 ± 0.246
Petit Rocher	Nigadoo River Intake	47.73718	-65.79547	n/a	5.665 ± 2.642	2.275 ± 0.165
Dalhousie	Charlo River	47.94791	-66.40395	0.168 ± 0.018	2.013 ± 0.000	0.945 ± 0.1
Dalhousie	Charlo River Intake	47.96541	-66.34644	0.45 ± 0.115	2.823 ± 0.665	0.313 ± 0.039
Bathurst	Middle River	47.55004	-65.94349	0.27 ± 0.058	4.46 ± 0.279	0.688 ± 0.173
Bathurst	Middle River Intake	47.61703	-65.67696	0.138 ± 0.027	7.423 ± 0.696	0.22 ± 0.029

APPENDIX 2: Mean (± standard error) concentrations of tebufenozide reported from water samples collected from the 2018 Early Intervention Strategy research area, reported as milligram per liter (mg/L).

Watershed	Site Name	Latitude	Longitude	+3days	+2weeks
Miramichi River	Miramichi West	47.19316	-66.03952	0.000008 ± 3.0×10^{-6}	0.000015 ± 5.0×10^{-6}
Miramichi River	Miramichi East	47.23099	-65.83933	0.00003 ± 4.0×10^{-6}	0

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