Healthy Forest Partnership “Early Intervention Strategy” report on water monitoring within the 2019 research areas for Foray® 76B *Bacillus thuringiensis* var. kurstaki (*Btk*) and Limit® 240LV (tebufenozide)

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BACKGROUND

In 2015, a water monitoring program was initiated by the Healthy Forest Partnership’s “Early Intervention Strategy” (EIS) to quantify deposits of *Btk* and tebufenozide following treatments used in the EIS project. The following results are from the 2019 water monitoring program.

PRODUCTS

Btk

Bacillus thuringiensis var. *kurstaki* (*Btk*) is 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 only toxic to specific groups of insects (e.g., feeding caterpillars) and is effective for managing spruce budworm. During treatments, *Btk* is deposited on spruce and fir forests by aerial application. It must be eaten by budworm larvae to cause mortality, which occurs after the protein crystals present in *Btk* are released by the strong alkalinity of the insect’s gut. Note that the guts of vertebrates are highly acidic and thus when ingested the *Btk* crystals simply pass harmlessly through the digestive system. The *Btk* crystals open channels in the insect gut, which allows BTK spores and other toxic stomach bacteria to invade the rest of the body, essentially causing blood poisoning and death (Henry, 2014).

After decades of 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). Studies on *Bt* variants indicated that non-target organisms are not affected in any measurable way by the bacteria, including non-target 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.

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Tebufenozide

Another product used to manage spruce budworm is Limit® 240LV (tebufenozide), which is a synthesized version of an important insect growth regulator that interrupts normal development, leading to death or sterility (Natural Resources Canada, 2016). As with *Btk*, tebufenozide must be eaten by larvae to be effective. During treatments, tebufenozide is deposited on spruce and fir forests via aerial applications. Larvae typically stop feeding almost immediately upon ingestion 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). In general, 90-95% of tebufenozide is deposited in the forest canopy and is relatively rainfast, meaning that it is not easily washed off by rainfall (Kreutzweiser & Nicholson, 2007; Sundaram, 1995). 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).

Studies 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).

SAMPLING PROTOCOL

Water samples were collected from the Bathurst Harbour watershed (Middle River and its intake site) (Fig. 1) and from the Miramichi River watershed (Miramichi North and South) (Fig. 2). The Bathurst watershed was 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 four days prior to initial treatment of *Btk*, 2) within two days following final treatment of *Btk* and tebufenozide, and 3) two weeks following final treatment for *Btk* and tebufenozide. These sampling periods generally follow procedures developed and used by SOPFIM for *Btk* monitoring during the past two decades. Detection of tebufenozide is done using methods developed by Kreutzweiser and Nicholson (2007). Water samples from all sampling sites were collected along the shore (4 samples at approximately 10 meter intervals).

Figure 1.
Bathurst Harbour watershed's water sampling sites and areas treated.

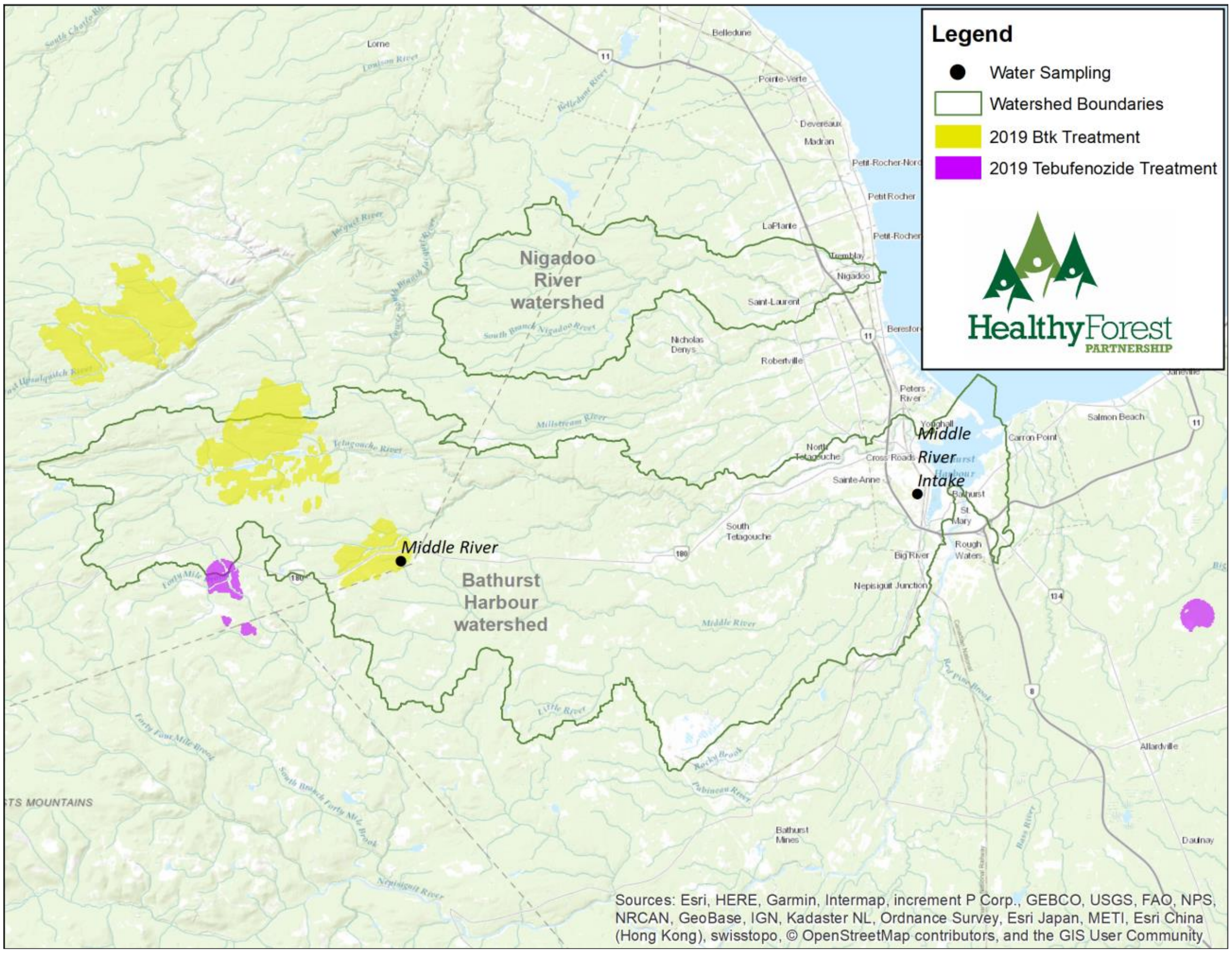
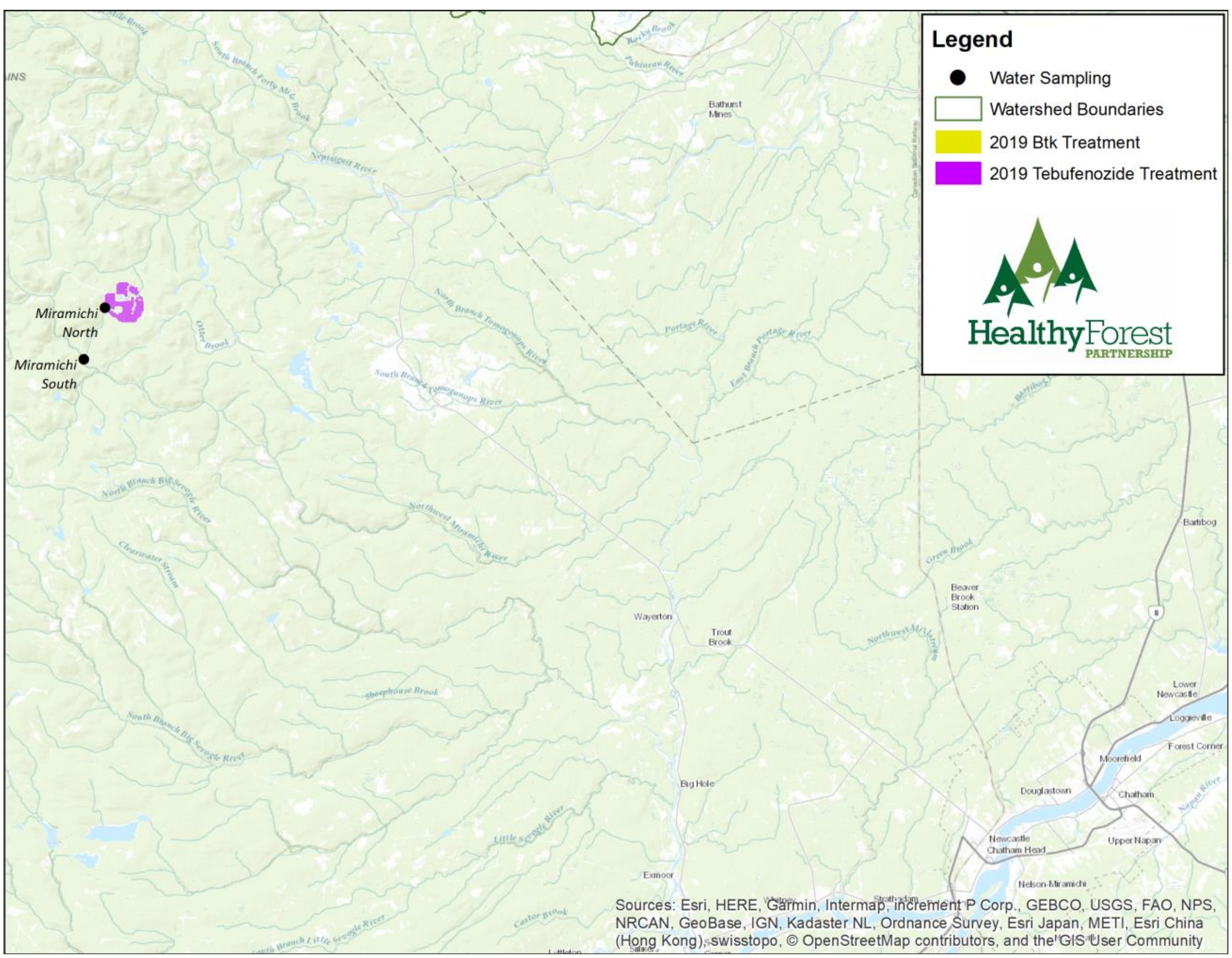


Figure 2.
Map of the Miramichi River watershed's water sampling sites and areas treated.



RESULTS AND DISCUSSION

Btk

Results for the Bathurst Harbor watershed water samples, analyzed by RPC in Fredericton, show that *Btk* concentrations increase from very low initial pre-treatment level to a maximum mean concentration of 234 CFU/ml at the Middle River sampling site 2 days following treatment and 0.013 CFU/ml at its intake sampling site. Two weeks following treatment, levels of Btk decrease to a maximum mean concentration of 8.083 CFU/ml at the Middle River site and 0.055 CFU/ml at its intake site (Appendix 1).

Tebufenozide

Results from laboratory analyses of the Miramichi Watershed water samples done by RPC in Fredericton, show trace levels of tebufenozide with a mean maximum concentration of 0.00006 mg/L at the Miramichi North sampling site 2 days following treatment and no detectable levels of tebufenozide at the Miramichi South sampling site. Two weeks following treatments, no tebufenozide was found at either site (Appendix 2).

CONCLUSIONS

Based on the results from this report, the 2019 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 2019 Early Intervention Strategy research area, reported as colony forming units per milliliter (CFU/mL).

Watershed	Site Name	Latitude	Longitude	Pre-treatment	+2days	+2weeks
Bathurst	Middle River	47.58094	-66.11467	3.985 ± 3.001	233.688 ± 113.775	8.083 ± 0.785
Bathurst	Middle River Intake	47.61702	-65.67747	0.095 ± 0.051	0.013 ± 0.008	0.055 ± 0.025

APPENDIX 2: Mean (± standard error) concentrations of tebufenozide reported from water samples collected from the 2019 Early Intervention Strategy research area, reported as milligram per liter (mg/L).

Watershed	Site Name	Latitude	Longitude	+2days	+2weeks
Miramichi River	Miramichi North	47.31143	-66.30953	0.00006 $\pm 4.0\text{x}10^{-6}$	0
Miramichi River	Miramichi South	47.28208	-66.32745	0	0

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