Healthy Forest Partnership report on water monitoring within the 2020 research areas for Foray® 76B *Bacillus thuringiensis* var. kurstaki (*Btk*) and 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 2020 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 foliage 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. The *Btk* crystals open channels in the insect gut, which allows *Btk* spores and other stomach bacteria to invade the rest of the body, essentially causing blood poisoning and death (Henry, 2014).

Note that the guts of vertebrates are highly acidic and thus toxic to Btk. After decades of testing, there is not a single instance of demonstrated toxicity to fish, mammals, birds, amphibians, or any aquatic organism. Studies on *Bt* variants indicate 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 EIS water monitoring project. Other non-target insect groups were reportedly unaffected at concentrations over 100,000 CFU/mL (EPA, 1998). The general lack of *Btk* toxicity for mammals 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 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 tebufenozide, which is a synthesized version of an important insect growth regulator that disrupts 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 even when present above normally applied levels found 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, Forty Four Mile Brook (Figure 1) and Kedgwick River watersheds (Figure 2). The Bathurst Harbour watershed was treated within or nearby the *Btk* treatment area and the Forty Four Mile Brook and Kedgwick River watershed were treated within or nearby the tebufenozide treatment area. Samples were collected at the intervals: 1) within one week 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 completed 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).

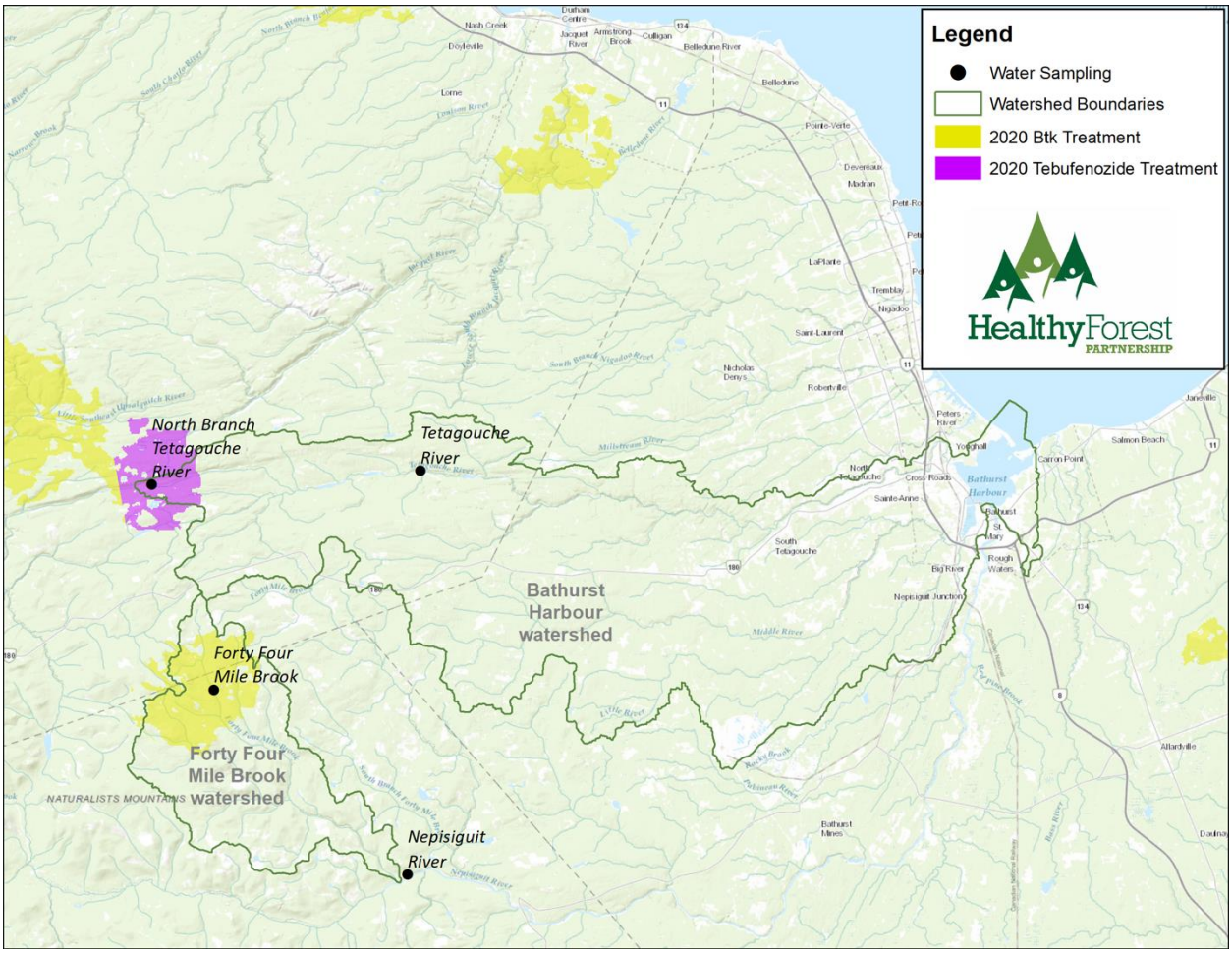

Figure 1. Map of the Bathurst Harbour and Forty Four Mile Brook watershed water sampling sites and areas treated.

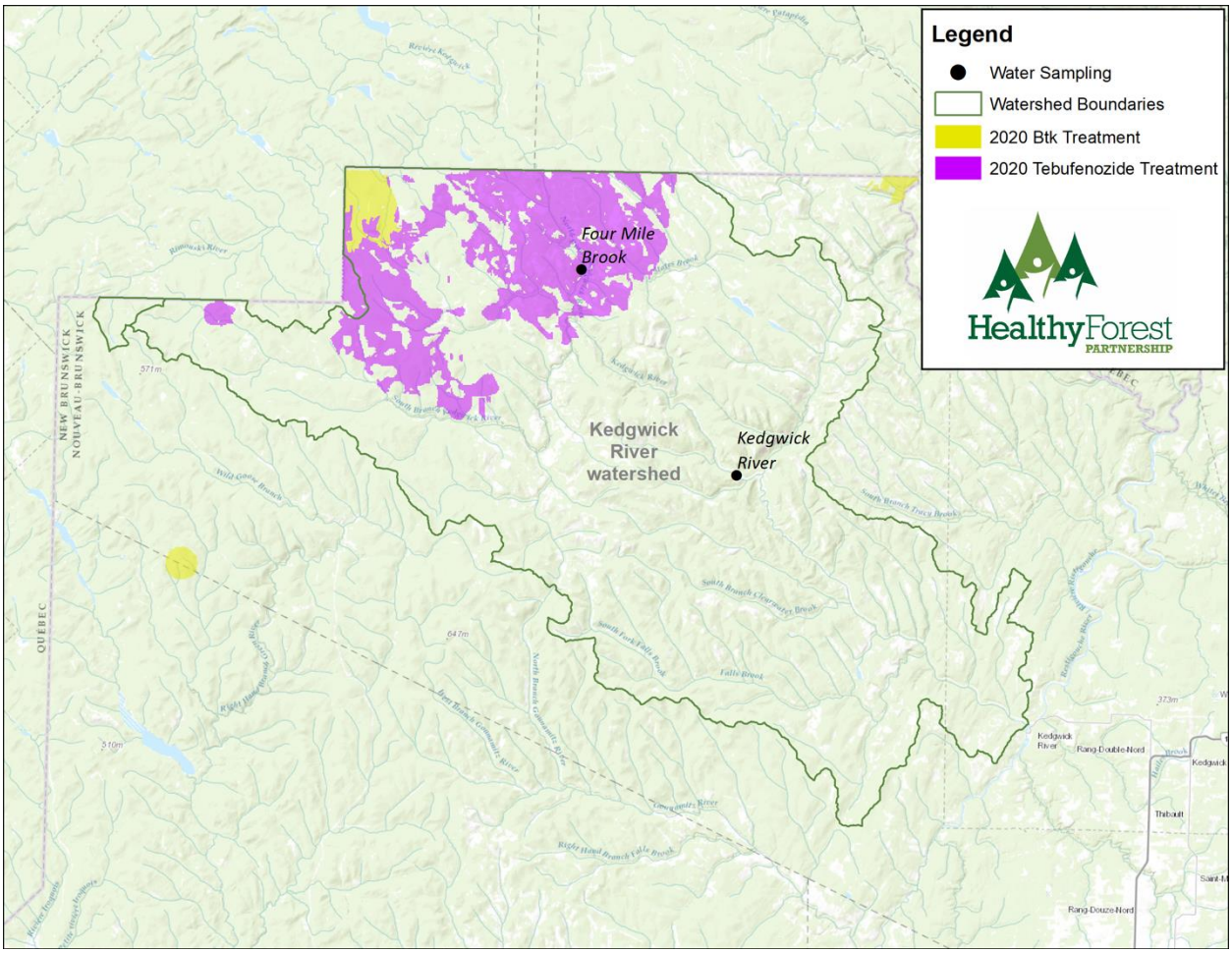

Figure 2. Map of the Kedgwick River watershed water sampling sites and areas treated.

RESULTS AND DISCUSSION

Btk

Results for the Bathurst Harbour 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 6.7 CFU/ml at the North Branch Tetagouche River sampling site two days following treatment and 1.415 CFU/ml at the Tetagouche River sampling site. Two weeks following treatment, levels of Btk decrease to a maximum mean concentration of 0.66 CFU/ml at the Middle River site and 0.153 CFU/ml at its intake site (Appendix 1).

Tebufenozide

Results from laboratory analyses of the Forty Four Mile Brook watershed water samples done by RPC in Fredericton, show trace levels of tebufenozide with a mean maximum concentration of 0.00007 mg/L at the Forty Four Mile Brook sampling site two days following treatment and no detectable levels of tebufenozide at the Nepisiguit River sampling site. Two weeks following treatments, 0.0006 mg/L of tebufenozide was found at the Forty Four Mile Brook and no tebufenozide was found at the Nepisiguit River sampling site. No tebufenozide was found in the water samples at the Kedgwick River watershed sampling sites (Appendix 2).

CONCLUSIONS

Based on the results from this report, the 2020 Early Intervention Strategy research treatments resulted in what are essentially trace levels of *Btk* and tebufenozide within and nearby watersheds.

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
Forty Four Mile	Forty Four Mile Brook	47.50980	-66.34987	0.11 ± 0.102	6.7 ± 2.7	0.66 ± 0.288
Forty Four Mile	Nepisiguit River	47.39618	-66.17475	0.01 ± 0.014	1.415 ± 0.97	0.153 ± 0.072

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
Kedgwick River	Four Mile Brook	47.93939	-67.90462	0.00007 ± $4.x10^{-5}$	0.00006 ± $8.2x10^{-6}$
Kedgwick River	Kedgwick River	47.81467	-67.75975	0	0
Bathurst Harbour	North Branch Tetagouche River	47.63616	-66.40583	0	0
Bathurst Harbour	Tetagouche River	47.64419	-66.16141	0	0

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