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Consequences of imidacloprid treatments for hemlock woolly adelgid on stream water quality in the southern Appalachians

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ABSTRACT

Imidacloprid, a neonicotinoid pesticide, is commonly used in hemlock woolly adelgid, *Adelges tsugae* (Annand) (HWA) (Hemiptera: Adelgidae), pest management programs to preserve hemlock resources. Great Smoky Mountains National Park (GRSM) has an extensive HWA integrated pest management program, with more than 200,000 individual hemlocks in the Park having received imidacloprid soil treatments. A retrospective study was conducted in cooperation with GRSM to assess imidacloprid and two of its insecticidal metabolites (5-hydroxy and olefin) are present in surface waters (i.e., streams) associated with HWA imidacloprid treatment areas.

Thirty stream locations were sampled in GRSM to assess the presence and concentration of imidacloprid, 5-hydroxy, and olefin. Water samples were collected from 10 streams downstream from riparian areas where hemlocks received imidacloprid soil treatments and immediately upstream from hemlock treatment areas in each of the selected 10 streams. In addition, water samples were collected from 10 control streams each in close proximity to one of the 10 streams flowing through treatment areas. The concentrations of imidacloprid, 5-hydroxy, and olefin in parts per trillion (ppt) were determined by liquid chromatography mass spectroscopy (LC/MS). Data analysis included historical treatment data from GRSM. Data were analyzed using a Kruskal–Wallis test ($P < 0.05$), least significant difference (LSD), and a multiple regression ($P < 0.05$).

Imidacloprid, in concentrations ranging from 28.5 to 379 ng L^{-1}, was detected in 7 of the 10 downstream sampling locations. Upstream or adjacent stream locations did not have detectable concentrations of imidacloprid. Five-hydroxy and olefin were not detected in any streams. A positive relationship between the total amount of imidacloprid applied to a hemlock treatment area and the concentration of detectable imidacloprid in the associated stream was observed. However, while imidacloprid was detected in streams associated with hemlock treatment areas, the concentrations are below USEPA chronic and acute aquatic life benchmarks for fish (1200 and 41,500 μg L^{-1}, respectively) and aquatic macroinvertebrates (1.05 and 34.5 μg L^{-1}, respectively). Since the amount of imidacloprid applied in a treatment area has an influence on the concentration of imidacloprid in streams, resource managers must carefully consider the frequency and extent of imidacloprid applications to meet management goals while providing minimal environmental impact.

1. Introduction

Hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae) (HWA), an invasive insect from southern Japan (Havill et al., 2006), was unintentionally introduced to the eastern United States in the 1950s (Stoetzel, 2002). HWA feeds on eastern hemlock, *Tsuga canadensis* (L.) Carriére, a slow-growing species that inhabits a distinctive ecological niche and is an important component of many forest types (Orwig and Foster, 1998; Ward et al., 2004). As the dominant shade-tolerant conifer in its habitat, eastern hemlock plays a vital ecological role in southern Appalachian forests, and that role cannot be filled by any other native evergreen tree species (Orwig and Foster, 1998; Ward et al., 2004). Many species depend on eastern hemlock and will be negatively impacted by its decline (Wallace and Hain, 2000; Hakeem, 2008; Dilling

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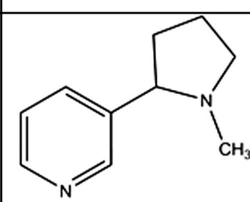
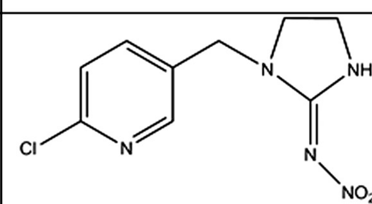
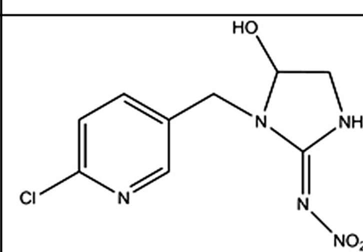
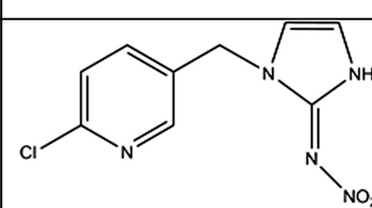
et al., 2007, 2009; Coots et al., 2012). Unfortunately, as eastern hemlock has exhibited no visible resistance against the adelgid (McClure, 1995) and no native predators are capable of suppressing adelgid populations (McClure, 1987), excessive mortality and decline have occurred throughout most of the natural range of this native tree species in the eastern United States (Lambdin et al., 2006).

Great Smoky Mountains National Park (GRSM) launched an aggressive integrated pest management (IPM) program against HWA to reduce damage to its hemlock resources once HWA was documented in the Park in 2002. Horticultural oil sprays, biological control (i.e., predatory beetles), and systemic imidacloprid applications have been employed to suppress HWA populations. Imidacloprid, a neonicotinoid pesticide, is the primary management tactic used in this program in the Park, where it is applied in GRSM as soil injections within 30 cm of the hemlock trunk, basal drench (i.e., imidacloprid solution is poured on the soil within 30 cm of the hemlock trunk), stem injections, or as a dissolvable pellet (Core Tect). Over 200,000 trees, many in riparian areas, have received imidacloprid soil treatments.

Imidacloprid has been used for pest control since the early 1990s (Diehr et al., 1991) and is applied in agricultural, forestry, and urban settings to suppress a variety of pest species (Jeschke et al., 2011; Goulson, 2013). The chemical structure of imidacloprid is similar to nicotine (Fig. 1) (Matsuda et al., 2001), and it functions similarly by acting on nicotinic acetylcholine receptors in the central nervous system of insects (Nauen and Bretschneider, 2002). Neonicotinoids are commonly used because they are selective for treating arthropod pests, have low fish and mammalian toxicity, and can be applied by various methods (Sánchez-Bayo and Hyne, 2014). However, concerns about the potential negative impacts of imidacloprid to surface water quality, aquatic macroinvertebrates, pollinators, and other non-target organisms have been expressed (USEPA, 2008b; Dilling et al., 2009; Pestana et al., 2009; Goulson, 2013).

Because imidacloprid can be toxic to aquatic macroinvertebrates if the dosage is high enough (Alexander et al., 2007; Pestana et al., 2009), its ability to leach into surface water and persistence in aquatic systems are important. Movement of imidacloprid through the soil is a route of potential impact to surface water quality (USEPA, 2008b). Similar to other pesticides, once in the environment, imidacloprid begins to degrade by biotic, abiotic, and photolytic degradation (Wamhoff and Schneider, 1999), and some degradation products of imidacloprid, such as olefin, 5-hydroxy, 4-hydroxy, and dihydroxy, have insecticidal properties (Nauen et al., 1998, 1999). The persistence of imidacloprid and its metabolites in the environment will influence their potential to cause negative non-target impacts.

The persistence of imidacloprid in the soil, determined by its ability to bind to soil and its degradation in the soil column (Cox et al., 2004), can affect which compounds enter surface waters. The sorption of imidacloprid into soil is dependent on the concentration of imidacloprid and the organic matter content in the soil, as imidacloprid binds to organic matter (Mullins and Christie,

Common Name	IUPAC Name	Structure
Nicotine	3-(1-methyl-2-pyrrolidinyl)pyridine	
Imidacloprid	1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine	
5-hydroxy	1-(6-chloro-3-pyridylmethyl)-2-(nitroimino)imidazolidin-5-ol	
Olefin	1-(6-chloro-3-pyridylmethyl)-*N*-nitro-1,3-dihydro-imidazol-2-ylideneamine	

[1]IUPAC = International Union of Pure and Applied Chemistry

Fig. 1. The IUPAC[1] names and chemical structures of nicotine, imidacloprid, and two insecticidal imidacloprid metabolites (5-hydroxy and olefin).

1995; Cox et al., 1998). In soils with high organic matter content, such as those in GRSM, less leaching is expected (Cox et al., 1998).

Once imidacloprid enters surface water its ability to persist may be limited because imidacloprid photodegrades in water (Moza et al., 1998; Wamhoff and Schneider, 1999). The half-life of imidacloprid in water has been recorded from one hour to three days (Agüera et al., 1998; Moza et al., 1998; Wamhoff and Schneider, 1999), and half-life can vary by season, ranging from estimates of 8.6–52.8 h, with slower photodegradation occurring in the winter (Lu et al., 2015). In the absence of light, imidacloprid is stable in water for more than 12 h. However, when exposed to light complete degradation has been documented in less than five hours (Agüera et al., 1998).

Possible non-target effects of imidacloprid in eastern hemlock systems in both terrestrial and aquatic habitats have been investigated by numerous researchers (Hakeem, 2008; Dilling et al., 2009; Churchel et al., 2011). Imidacloprid applied to hemlocks by soil injection can move laterally and horizontally through the soil (Knoepp et al., 2012). In numerous studies imidacloprid has been documented in surface waters associated with soil applications of imidacloprid in agricultural areas (Starner and Goh, 2012; Hladik et al., 2014; Main et al., 2014). Imidacloprid and its metabolites may move into the water column through leaf degradation, since imidacloprid, olefin and 5-hydroxy have been detected in hemlock foliage tissue (Dilling et al., 2010; Coots et al., 2013). A similar scenario has been documented in the laboratory using ash leaves, where imidacloprid was found to enter the water column as leaves from treated ash trees degraded (Kreutzweiser et al., 2007). Given the presence of imidacloprid in surface waters via various routes, imidacloprid treatments for hemlock conservation may pose potential risks to surface water quality. The purpose of this study is to assess the potential risks of imidacloprid in surface waters in GRSM by determining the presence and concentration of imidacloprid, 5-hydroxy, and olefin in surface waters and if any treatment area and timing factors contribute to observed concentrations of the insecticidal chemicals in water.

2. Materials and methods

Ten streams flowing through hemlock-dominant or co-dominant forest types in treatment areas were selected for this study (Table 1). Ten locations, one in each stream, were selected 10–100 m downstream from a treatment area, hereafter referred to as downstream. As a control, ten locations, one in each stream, were selected 10–100 m upstream from the treatment areas, hereafter referred to as upstream. In addition, ten streams were selected in hardwood-dominant forest types, in the same watersheds as the streams in treatment areas, and are henceforth referred to as adjacent streams. No imidacloprid treatments were applied upstream from the adjacent stream locations; thus, these locations serve as an additional control. Water samples were collected from 30 stream locations (10 upstream, 10 downstream, and 10 adjacent stream) (Fig. 2) in GRSM to assess the presence and concentration of imidacloprid and two of its metabolites (5-hydroxy and olefin) (Fig. 1).

Treatment areas contained between 100 and 1000 hemlocks that received imidacloprid treatments. Hemlocks in the riparian corridors of treatment areas were treated one to eight years before sampling and received between one and three treatment cycles, depending on the site (Table 1). A treatment cycle may refer to a time when most trees in a treatment area were treated or when the hectarage of a treatment area was expanded. Due to hemlock health in selected treatment areas and the expansion or contraction of the size of treatment areas, the number of trees per treatment area was not consistent among treatment cycles. For example, a larger treatment area may have had many treated hemlocks initially, but with hemlock mortality due to HWA in that area, fewer trees would have been treated during the next cycle. A few trees near campsites also may have had an initial treatment and later the larger area around the campsite was treated.

Imidacloprid was applied as a basal drench, which involves pouring a wettable powder solution of imidacloprid around the base of hemlock trees approximately 30 cm from the trunk. Trees smaller than 25 cm diameter at breast height (dbh) were treated with 0.7 g.a.i. (grams of active ingredient) per 2.5 cm dbh, and trees 25 cm dbh and larger were treated with 1.4 g.a.i. per 2.5 cm. Imidacloprid rates per hectare did not exceed the maximum allowable rate of treatment (0.4 kg per hectare per year) (Bayer, 2006).

Samples were collected from each selected location (either upstream, downstream, or adjacent stream) during a single sampling event. During a sampling event three replicate water samples (1 L) were collected mid-channel and mid-depth from each stream sampling location using amber glass bottles (1 L) with Teflon lined lids. Glass bottles were placed into the water column, lid down. The bottle was then turned with the opening facing upstream to allow the bottle to fill with stream water. Containers were transported to and from the field in cooler bags (25 × 15 × 15 cm). Sampling locations were often in remote areas, so the cooler bags

Table 1
Imidacloprid treatment histories for streams in treatment areas where imidacloprid was used for the management of hemlock woolly adelgid, Great Smoky Mountains National Park.

Stream	First treatment	Last treatment	Sampling date	Treated hectares	Total kg.a.i.[a,b] applied	kg.a.i. 1 yr prior to sampling[c]	Treated stream length (m)	Treatment cycles
Alum Creek	9/2004	8/2011	6/2012	19.0	14.8	0.2	4008	5
Camel Hump Creek	N/A[d]	N/A	5/2012	N/A	N/A	1.2	353	N/A
Cane Creek	2/2005	10/2010	2/2013	14.5	6.3	0	4178	3
Chasteen Creek	1/2005	6/2009	12/2012	42.6	16.8	0	8766	4
Dunn Creek	4/2005	9/2010	6/2012	47.1	114.0	0	1046	6
Indian Camp Creek	5/2005	9/2010	6/2012	N/A	N/A	0	9899	N/A
Indian Creek	9/2005	6/2011	8/2012	47.2	38.3	0	5046	5
Kingfisher Creek	5/2004	10/2012	10/2012	29.4	20.9	9.7	1773	4
Panther Creek	8/2011	4/2012	8/2012	26.6	1.8	1.8	3811	1
Shop Creek	4/2011	6/2011	10/2012	23.3	7.6	0	2249	1

[a] Kilograms active ingredient.
[b] Total kg.a.i. applied in the treatment area.
[c] kg.a.i. applied in the treatment area one year before water samples were collected.
[d] All data were not available for Camel Hump Creek and Indian Camp Creek.

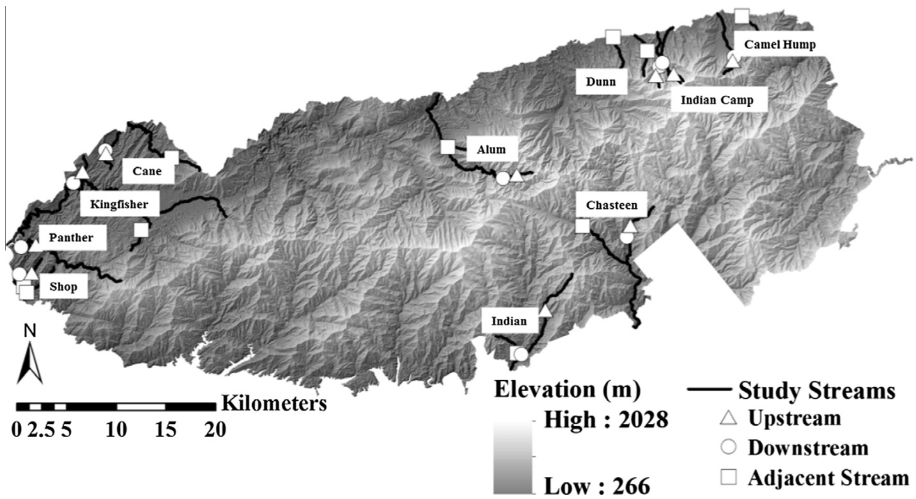


Fig. 2. Stream sampling locations in the Great Smoky Mountains National Park.

were placed in large backpacks for transport to the laboratory, where samples were stored in a walk-in cooler at 4 °C until processed. Samples were processed within three weeks of the collection date. All samples were collected between May 2012 and January 2013.

The amount of sample collected was sufficient to allow for concentration detection in parts per trillion (ng L^{-1}). All methods were optimized for greater sensitivity to determine low levels of imidacloprid in the environmental samples using liquid chromatography mass spectroscopy (LC/MS). Sample preparation prior to analysis utilized an Empore aqueous extraction system (Mueller et al., 2000; Mersie et al., 2002; Senseman et al., 2003). This procedure passes the water sample through a 17 mm C 18 embedded filter allowing the matrix to pass through unimpeded and capturing the analytes of interest, imidacloprid, 5-hydroxy, and olefin. Preliminary studies examined the recovery of fortified imidacloprid samples using our methodology, and indicated that recovery was 49.0–52.5% (data not shown). Repeated attempts to increase recovery trying a range of different solvents and operating parameters were not successful. While recovery in the import system of 49.0–52.5% was not ideal, the consistency and relative goodness of that 50% across several validation runs encouraged the use of the described procedures. In addition, the Empore solid phase extraction platform is widely recognized as an appropriate sample processing and concentrating procedure. Thus, the determination of concentration recovery in our samples was 50%.

The entire water sample (1 L) was passed through an Empore disk (3 M) on an Empore 6 station extraction manifold and processed using standard laboratory procedures to prepare a given water sample for LC/MS analysis. First, the Empore disk was conditioned using methanol. Once the disk was conditioned, the water sample was added to a reservoir, which holds the water above the disk. Water was then drawn through the disk using a vacuum pump (GAST model P104 oil-less pump) operated at 0–7 bar of negative pressure. Residual water was removed from the disk using the vacuum pump to dry the disk. The sample was eluted using 10 mL of methanol and collected in a 12 mL vial. This sample preparation resulted in a highly concentrated sample that was prepared for LC/MS. Processed samples were stored at 4 °C until LC/MS analysis.

Chromatographic conditions included use of the C 18 column (Phenomenex, Inc.) and isocratic mobile phase of 30% acetonitrile and 70% water (both with 0.1% formic acid to maintain constant ionic strength). Mass spectroscopy conditions included drying gas flow of 5.0 L, nebulizer pressure at 4.14 bar, drying gas temperature of 300 °C, vapor temperature 250 °C, capillary voltage 2000 V, Corona current was set at 1.0, charging voltage was set at 2000, and the fragmentor setting was 70. The ionization hardware used was mixed mode-ESI–APCI. Apparent molecular mass units using the select ion monitoring mode determined the imidacloprid, 5-hydroxy, and olefin simultaneously. Approximate retention times for imidacloprid, 5-hydroxy, and olefin were 8.50, 5.98, and 5.26 min, respectively.

They were analyzed as a group and each run included individual standards for the parent and metabolites, with an external standard technique used. The conservative limit of detection (LOD) was 20 ng L^{-1}. Given the difficulty of collecting and storing samples, due to remote site location, the decision was made not to collect blank water samples or fortify deionized water samples in the field. Method development strongly indicated that procedures used in this study were robust and highly precise for the detection and quantification of the target compounds.

Rainfall data were obtained from the National Oceanic and Atmospheric Administration (NOAA) climate data website (NOAA, 2015). NOAA weather stations closest to the watersheds of interest were used. Data three days prior to sample collection were used to determine how much rainfall had recently fallen in the sampled watersheds. Data were not used in the analyses because rainfall records were not available for Camel Hump Creek and incomplete for Cane Creek and Chasteen Creek.

All data were stored using an Excel file (Microsoft, Redmond, WA). The three replicate samples collected at each sampling location were averaged, to obtain one concentration for each sampling location for use in data analyses. A Kruskal–Wallis Test was used to determine significant differences, if any, among ranks of concentration of imidacloprid found in upstream, downstream, and adjacent stream sampling locations ($P < 0.05$). The mean ranks were separated using least significant difference (LSD). A multiple regression analysis was used to determine if a relationship existed between treatment area information and time variables and the concentration of imidacloprid documented in streams in GRSM ($P < 0.05$). A backward elimination selection method was used to select the model that best explained the data. All data analyses were conducted using SAS (SAS Institute, 2008). The Camel Hump Creek treatment area was never isolated as a separate site from a larger treatment area in regards to data entry, so accurate numbers on treatment time and site variables specifically to that smaller watershed are not available. Indian Camp Creek flows through numerous treatment areas, but does not have a distinct treatment drainage area for treatment time and site variables. Because all site data are not available for Camel Hump Creek and Indian Camp Creek they were not included in the multiple regression analysis.

3. Results

Imidacloprid was detected in streams associated with imidacloprid treatments for the control of HWA in this study (Table 2). All stream locations where imidacloprid was detected were downstream from imidacloprid treatment areas. Imidacloprid was detected in seven out of ten downstream locations, and imidacloprid concentrations ranged from 28.5 to 379.1 ng L^{-1}. In six of the streams where imidacloprid was detected the concentration of imidacloprid was below 100 ng L^{-1}. Dunn Creek, with a documented imidacloprid concentration of 379.1 ng L^{-1}, was the only stream where the concentration of imidacloprid was in excess of 100 ng L^{-1}. Three downstream locations, Camel Hump Creek, Cane Creek, and Panther Creek, had no samples that exceeded the LOD for imidacloprid. Samples from all upstream and adjacent stream locations did not exceed the LOD for imidacloprid (data not shown). All samples were below the LOD for 5-hydroxy and olefin (data not shown).

Rainfall amounts and imidacloprid concentrations detected in streams do not have a clear pattern, which may be, in part, due to the variety of treatment area conditions in the study. The two highest concentrations recorded, 379.1 and 78.0 ng L^{-1}, were detected in Dunn Creek and Indian Creek, respectively. Nearly 1 cm of rainfall occurred three days prior to sample collection, which may have influenced the observed concentrations. However, rainfall events in excess of 2 and 3 cm occurred before samples were collected at Alum Creek and Indian Creek, respectively. While imidacloprid was detected in those streams, concentrations were only 28.5 and 31.2 ng L^{-1}, respectively. Little to no rain occurred prior to sampling at Panther Creek, Chasteen Creek, and Kingfisher Creek. Imidacloprid was detected at both Kingfisher Creek (33.6 ng L^{-1}) and Chasteen Creek (36.8 ng L^{-1}). Given the diversity of hectarage and imidacloprid usage in treatment areas, it would be difficult to perceive overall trends in imidacloprid concentrations in stream water based on rainfall.

A significant difference among upstream, downstream, and adjacent stream categories was detected ($X^2 = 52.92$, df = 2, $P < 0.001$; Kruskall–Wallis). Downstream locations have a significantly higher mean rank for imidacloprid concentrations than upstream and adjacent stream locations ($P < 0.05$; LSD test). Locations downstream from imidacloprid treatment areas had significantly higher concentrations of imidacloprid than upstream and adjacent stream locations, both of which did not have detectable concentrations of imidacloprid.

The selected multiple regression model, which includes months since the first and last imidacloprid treatments, the number of treated hectares, and the total amount of imidacloprid applied to treatment areas, explains 97% of the variation in the data. The model overall was significant ($P = 0.009$), and all variables could explain at least 48% of the variation adjusted for the other variables. Given the adjustments made for the other variables in the model, the concentration of imidacloprid found in streams is positively related to the total amount of imidacloprid applied to treatment areas (Partial $R^2 = 0.96$, $P = 0.002$) (Fig. 3 and Table 3). Cane Creek and Panther Creek, two sites where imidacloprid was not detected, had the smallest amounts of imidacloprid applied to their treatment areas, 6.3 and 1.8 kg.a.i., respectively. Dunn Creek, which had an imidacloprid concentration of 379.1 μg L^{-1}, also received the greatest amount of imidacloprid applied to the treatment area (114.0 kg.a.i.). The concentration of imidacloprid detected at Dunn Creek is largely responsible for the slope of the relationship between the concentration of imidacloprid and the amount of imidacloprid applied to treatment areas. No significant relationship was detected between imidacloprid concentrations and treatment area variables when Dunn Creek was removed from the analysis and only lower concentration data points were considered (data not shown). However, data collected from Dunn Creek are valid and explain much of the relationship between imidacloprid concentration in streams and the amount of imidacloprid applied in treatment areas (Table 3).

4. Discussion

The potential risk of imidacloprid from hemlock treatments to leach through soil, enter surface water, and cause associated negative impacts on water quality and aquatic biota is an issue that scientists, regulators, and land managers must consider. According to the USEPA, the Chronic and Acute Aquatic Life Benchmarks of imidacloprid for fish is 1200 and 41,500 μg L^{-1}, respectively. Aquatic invertebrates have much lower Chronic and Acute Aquatic Life Benchmarks of 1.05 and 34.5 μg L^{-1}, respectively (USEPA, 2008a). The LC_{50} (the concentration at which 50% of individuals of a taxa are killed) of imidacloprid for aquatic macroinvertebrates in 96 h exposure studies ranges from 0.65–12.94 μg L^{-1} (Alexander et al., 2007; Stoughton et al., 2008; Pestana et al., 2009). Sublethal effects on aquatic macroinvertebrates have been documented at

Table 2
Concentration in ng/L (parts per trillion) of imidacloprid at downstream locations and rainfall totals three days prior to sample collection, Great Smoky Mountains National Park.

Stream name	Imidacloprid concentration[a]	Rainfall (cm)
Alum Creek	28.5 ± 3.8	2.44
Camel Hump Creek	<LOD[b]	na[c]
Cane Creek	<LOD	0.53[d]
Chasteen Creek	36.8 ± 3.4	0[d]
Dunn Creek	379.1 ± 7.9	0.97
Indian Camp Creek	78.0 ± 8.0	0.97
Indian Creek	31.2 ± 1.5	3.35
Kingfisher Creek	33.6 ± 6.6	0
Panther Creek	<LOD	0.20
Shop Creek	82.2 ± 25.8	0.71

[a] Means (± standard deviation) are an average of the concentrations of the three samples collected at each sample location.
[b] Imidacloprid concentration was below the limit of detection (LOD) (20 ng/L).
[c] Rainfall data for Camel Hump Creek were not available.
[d] Complete rainfall data were not available during the three day time period prior to sampling.

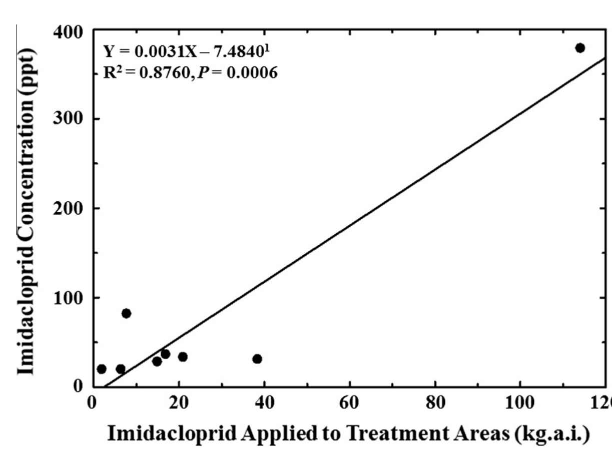


Fig. 3. Relationship between the amount of imidacloprid applied to treatment areas and concentration of imidacloprid observed in streams. [1]No adjustments are made for other variables.

Table 3
Multiple regression associating imidacloprid concentration in streams with treatment area information and time variables.

Variable	DF	Parameter estimate	Standard error	*t* Value	Pr > |t|	Partial R^2
Intercept	1	99.3703	36.8975	2.69	0.074	–
Mo. Since First Treatment	1	−0.9091	0.3260	−2.79	0.069	0.7216
Mo. Since Last Treatment	1	1.4638	0.8674	1.69	0.190	0.4870
Hectares	1	−2.8644	1.1769	−2.43	0.093	0.6638
Total kg.a.i. Applied[a]	1	0.00402	0.0004	9.72	0.002	0.9694

[a] Total kg.a.i. applied in the treatment area.

concentrations of 0.10–3.00 μg L^{-1} in 96 h exposure trials (Azevedo-Pereira et al., 2011; Roessink et al., 2013). Sublethal effects of imidacloprid were observed in a mesocosm experiment using 12 μg L^{-1} imidacloprid pulses simulating rainfall event frequency and duration (Mohr et al., 2012).

Short-term exposure data are currently used to set water quality standards. Because the cumulative effect of exposure to low imidacloprid concentrations may have sublethal impacts on aquatic macroinvertebrates, concern has been raised regarding the use of these methods. In addition, the USEPA Aquatic Life Benchmark concentrations are higher than standards set by Canada, Europe, and the Netherlands (Morrissey et al., 2015). While negative effects of imidacloprid exposure on aquatic macroinvertebrates have been documented, most concentrations observed in this study are below concentrations documented to have negative acute and chronic effects. Six streams had documented imidacloprid concentrations that were less than 0.10 μg L^{-1}, which is one tenth of the USEPA Chronic Aquatic Life Benchmark. Dunn Creek was the only sampling location where imidacloprid concentration was in excess of 100 ng L^{-1}, and the observed concentration (379.1 ng L^{-1}) is below the above-mentioned USEPA Chronic and Acute Aquatic Life Benchmarks of imidacloprid for both aquatic macroinvertebrates and fish. In addition, preliminary results from a complementary study assessing aquatic macroinvertebrates in the upstream and downstream locations indicate similar abundance and taxa richness of environmentally sensitive aquatic macroinvertebrate taxa (unpublished data). Examination of the aquatic community composition among sites will be addressed in a separate publication.

Imidacloprid has been previously documented in a stream associated with imidacloprid treatments for suppression of HWA. In that study, four streams were sampled for approximately 2 yr after imidacloprid soil applications, and only one sample, collected over 700 d after treatment, tested positive for imidacloprid. The concentration of imidacloprid in the only positive sample was <1 μg L^{-1}. However the LOD for their study was 0.6 μg L^{-1} (Churchel et al., 2011), which is 30 times higher than the LOD in the current study. All documented concentrations of imidacloprid in our study were lower than the 0.6 μg L^{-1} LOD in Churchel et al. (2011). If methods used in that study had allowed for a lower LOD, then more positive samples may have been detected in streams associated with imidacloprid treatments for HWA. In addition to low documented presence of imidacloprid in streams, no negative effects on aquatic macroinvertebrates were observed in the stream where imidacloprid was documented (Churchel et al., 2011).

The absence of olefin and 5-hydroxy in stream samples was not unexpected. Olefin and 5-hydroxy are not the main metabolites of imidacloprid produced via photodegradation in water (Agüera et al., 1998; Moza et al., 1998; Redlich et al., 2007). However, since both metabolites are highly toxic insecticidal metabolites produced in numerous plant systems, including hemlock, it is important to establish the absence of olefin and 5-hydroxy in streams flowing through HWA treatment areas (Nauen et al., 1998, 1999; Coots et al., 2013).

Eastern hemlock is an important component of southern Appalachian riparian ecosystems with many aquatic and terrestrial species depending on its presence. With hemlocks in eastern forests declining, land managers must make difficult decisions involving positive and negative trade-offs of treatments for the protection of hemlock resources. Assessment of the presence and concentration of imidacloprid in streams as a result of imidacloprid treatments to hemlocks is an initial step to determine what negative consequences to surface water quality must be considered when making management decisions. Because the amount of imidacloprid applied in a treatment area has a significant effect on the concentration of imidacloprid observed in streams in this study, the frequency and extent of imidacloprid applications must be carefully considered. Land managers must decide if the risk of imidacloprid exposure to aquatic macroinvertebrates adjacent to areas of treated hemlock outweighs the benefits of preserving hemlock, which is a key species in many systems.

5. Conclusions

Imidacloprid was present downstream from imidacloprid treatment areas in seven of ten streams, and the presence of imidacloprid was not observed in upstream and adjacent stream samples. The highest concentration observed, 379.1 ng L^{-1}, was below USEPA Aquatic Life Benchmarks for chronic toxicity of imidacloprid to aquatic invertebrates. Six of the seven streams where imidacloprid was documented had concentrations below 100 ng L^{-1}, less than one tenth of the USEPA Chronic Aquatic Life Benchmark. No obvious trends existed between the amount of rainfall prior to sampling and the observed concentration of imidacloprid in streams. A positive relationship between the total amount of imidacloprid that was applied in treatment areas and the imidacloprid concentration in streams was documented. Months since the first and last imidacloprid treatments as well as hectares treated explained at least 48% of the observed variation in imidacloprid concentration data. The insecticidal metabolites olefin and 5-hydroxy were not documented in any of the sampled streams. Knowledge about the presence and concentration of imidacloprid in multiple streams associated with HWA treatment areas can help land managers make calculated assessments of the risks and benefits of treating hemlocks with imidacloprid for the suppression of HWA. Based on these results, imidacloprid does appear in streams associated with HWA treatment areas. Concentrations detected are below USEPA Chronic and Acute Aquatic Life Benchmarks.

While chronic, sublethal effects are possible (Morrissey et al., 2015), according to guidelines currently set by the USEPA, detected imidacloprid concentrations are not expected to impact the aquatic community.

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