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Factors influencing the occurrence and distribution of neonicotinoid insecticides in surface waters of southern Ontario, Canada

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HIGHLIGHTS

- Conducted a survey of neonicotinoids used in full range of agricultural activities in surface waters of Ontario.
- Statistical correlation of individual compounds with land use was investigated.
- Relationship between neonicotinoid occurrence and hydrology of water courses was assessed.
- Imidacloprid, clothianidin, and thiamethoxam detection frequency over 90% at over half the sites sampled.
- At 2 sites, the Canadian freshwater guideline value for imidacloprid (230 ng/L) was exceeded in 75% of samples.

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ABSTRACT

The widespread use of neonicotinoid insecticides and recent increased regulatory scrutiny requires the generation of monitoring data with sufficient scope and resolution to provide decision makers with a better understanding of occurrence and distribution in the environment. This study presents a wide-scale investigation of neonicotinoid insecticides used across the range of agricultural activities from fifteen surface water sites in southern Ontario. Using statistical analysis, the correlation of individual compounds with land use was investigated, and the relationship between neonicotinoid occurrence and hydrologic parameters in calibrated water courses was also assessed. Of the five neonicotinoids studied, imidacloprid, clothianidin and thiamethoxam exhibited detection rates above 90% at over half the sites sampled over a three year period (2012–2014). At two sites in southwestern Ontario, the Canadian Federal freshwater guideline value for imidacloprid (230 ng/L) was exceeded in roughly 75% of the samples collected. For some watersheds, there were correlations between the occurrence of neonicotinoids and precipitation and/or stream discharge. Some watersheds exhibited seasonal maxima in concentrations of neonicotinoids in spring and fall, particularly for those areas where row crop agriculture is predominant; these seasonal patterns were absent in some areas characterized by a broad range of agricultural activities.

1. Introduction

In the past decade, the use of organophosphorous insecticides has been superseded by neonicotinoid insecticides (Hladik et al., 2014; Hladik and Kolpin, 2015; Morrissey et al., 2015; Anderson et al., 2015). Neonicotinoids are active against a wide range of insects, are effective at low concentrations, are systemic, and can be applied using a variety of methods (Anderson et al., 2015). Registered uses of neonicotinoids in Canada include control of insects on field and greenhouse crops, orchards and nurseries, woodlots, flea control on household pets, and control of turf pests in urban areas, sod farms and golf courses. Neonicotinoids are regulated nationally by Health Canada's Pest Management Regulatory Agency (PMRA) with additional provincial restriction under the Province of Ontario's 2009 ban on cosmetic use of pesticides on lawns and gardens under the Ontario Pesticides Act (Ontario, 2016). Neonicotinoid formulations are also used for seed treatment of row crops such as corn, soybeans and canola, which has led to widespread use in Ontario (McGee et al., 2010; Farm and Food Care

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Ontario, 2016).

There has been growing concern about use of neonicotinoid pesticides and possible ecological and ecotoxicological effects on pollinators and invertebrates, and possible indirect effects on songbirds and waterfowl (Anderson et al., 2015). Anderson et al. (2015) recently reviewed fate, exposure and biological effects of neonicotinoids in the Canadian aquatic environment, while Morrissey et al. (2015) reviewed neonicotinoid contamination in surface waters globally and potential risk to aquatic invertebrates. The United States Geological Survey (USGS) has conducted both national- (Hladik and Kolpin, 2015) and regional-scale (Midwestern United States, Hladik et al., 2014) reconnaissance studies of neonicotinoids in streams in the United States. Imidacloprid, clothianidin and thiamethoxam were the most frequently detected; in the U.S. national study, clothianidin and thiamethoxam were positively correlated with percentage of land use in cultivated crop production, while imidacloprid was positively correlated with percentage of urban area (Hladik and Kolpin, 2015).

To make informed decisions with respect to use, registration, and effects guidelines, there is a requirement for knowledge of occurrence and distribution of neonicotinoid insecticides across jurisdictions. The purpose of this study was to assess occurrence and distribution of neonicotinoids in surface waters in different agricultural and urban areas of southern Ontario as part of a comprehensive pesticide monitoring program. The neonicotinoids analyzed were thiamethoxam, clothianidin, imidacloprid, thiacloprid and acetamiprid; registration for the first three compounds is currently being re-evaluated by the PMRA. Morrissey et al. (2015) identified a scarcity of neonicotinoid insecticide data globally that enables inferences regarding the fate of these compounds in relation to water body features and land use. Using statistical analysis, the correlation of individual compounds with land use was investigated and the relationship between neonicotinoid occurrence and hydrologic parameters in calibrated water courses. This study presents the first wide-scale investigation of neonicotinoid insecticides in surface waters across the range of agricultural activities in southern Ontario.

2. Methods

Fifteen sites in southern Ontario consisting of nine streams near agricultural areas (drainage area <100 km^2), and six larger streams/rivers (drainage area >100 km^2) were sampled (Fig. S1). These stream sites reflected a range of agricultural activities including row crops, fruits and vegetables, orchards and grapes, greenhouses, ornamental nurseries, and turf. The sites also included an urban stream (Indian Creek) and a reference stream (Spring Creek) located adjacent to a national park removed from agricultural activities. All neonicotinoid insecticide concentrations in samples from Spring Creek were below the method detection limits (Table S1). Precipitation was sampled at one additional site (Bear Creek).

2.1. Sampling methods

Whole water samples were collected by submersing sample bottles (1L amber glass with Teflon® lids) at mid-stream to a depth of 10–20 cm, and stored in coolers with ice packs for transport. Samples were collected bi-weekly through the growing season (May–September) with monthly sampling in April, October, November and December. Duplicate field samples and field blanks were collected for QA/QC purposes. General water quality characteristics including temperature, pH, conductivity and dissolved oxygen were also measured during each sampling event using a YSI® sonde.

2.2. Sample preparation

Surface water and precipitation samples (800 mL stored at 4 °C) were extracted at 5 mL/min using a Waters OASIS HLB (0.5 g) solid phase extraction (SPE) cartridge. The cartridge was rinsed with 5 mL of 5% methanol in water (v/v) and then dried on-line with nitrogen for 1 min. The cartridge was eluted with 10 mL of methanol at a flow rate of 2 mL/min. The final extract was concentrated to ~0.9 mL and 50 μL of internal standard (acetamiprid-d_3 at 0.97 μg/mL, imidacloprid-d_4 at 1.3 μg/mL and thiamethoxam-d_3 at 1.0 μg/mL) was added and the extract volume-adjusted with water to a 1.5 mL final volume.

2.3. Analysis

The five neonicotinoids analyzed were acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam. An Agilent 1100 series HPLC system equipped with a Phenomenex Synergi Hydro-RP analytical column (3 × 100 mm i.d., 2.5 μm particle size) was used at a column temperature of 40° C and mobile phase flow rate of 250 μL/min. The mobile phase solvents were water (A) and 90% methanol (v/v) in water (B), each containing 5 mM ammonium formate used in a gradient elution program; initial composition 90% A:10% B; 90% A:10% B at 0.1 min; 5% A:95% B at 5.0 min and then held for duration of the 12 min run. The column was equilibrated for 5 min between 5 μL sample injections.

Neonicotinoid compounds were analyzed using an Applied Biosystems/Sciex API 2000 tandem mass spectrometer (MS) using an electrospray ionization (ESI) source in positive ion mode. The optimized positive ESI-MS conditions were; curtain gas (CUR) 35 psi, collision gas (CAD) 4 psi, TurboIon Spray source voltage (IS) 3000 v, heated nebulizer temperature 500° C, nebulizing gas (GS1) at 80 psi and auxiliary/heater gas (GS2) at 80 psi. The dwell time for each ion-pair was 50 ms. Resolution was set to achieve unit mass resolution for quadrupoles 1 and 3.

2.4. Statistical analysis

Summary statistics were estimated using the Kaplan-Meier method to account for values below detection limits using the NADA package in R (Helsel, 2012; R Core Team, 2016). Principal components analysis (PCA) was used to identify relationships between land-use, crop type, and neonicotinoid concentrations (Helsel and Hirsch, 2002). Prior to analysis by PCA all data were transformed to standard scores (z-score) as variables included have different units of measure. Association of individual neonicotinoids and association with precipitation and stream discharge were assessed using the Kendal rank correlation coefficient (Kendall's tau, τ). The PCA and correlation analyses were performed using JMP® Version 10 (SAS Institute Inc., Cary, NC).

3. Results and discussion

3.1. Occurrence and distribution of neonicotinoid insecticides in southern Ontario surface waters

As observed in other North American studies of neonicotinoids, imidacloprid, thiamethoxam and clothianidin were the most ubiquitous; occurrence and distribution data including surface water concentrations and frequency of detection for southern Ontario surface waters are shown in Table S1 and Fig. S2. Table S1 also includes the number of samples that exceeded the Canadian Council of Ministers of the Environment (CCME) interim freshwater guideline for protection of aquatic life value for imidacloprid (230 ng/L, CCME, 2007); this guideline is currently the only

Canadian federal freshwater guideline available for any neonicotinoids registered for use in Canada, and was used as a benchmark to compare with observed concentrations in this study.

Registered uses of imidacloprid in Canada include control of insects on field and greenhouse crops, orchards and nurseries, and household and turf applications (PMRA, 2001, 2016). In Ontario, imidacloprid replaced diazinon for lawn care use and turf applications (Struger and Fletcher, 2002), prior to the Province of Ontario's 2009 ban on cosmetic use of pesticides on lawns and gardens. Imidacloprid is applied to control insects across the entire range of agricultural activities using a variety of application methods including soil application, foliage spray treatment, and seed treatment (PMRA, 2001, 2016). Typical application rates to foliage or soil range are determined by crop, but typically range from 42 to 480 g a.i./ha (PMRA, 2016). However, the application rate for imidacloprid on fruiting vegetables for control of the Colorado potato beetle and aphids can be as high as 560 g a.i./ha (PMRA, 2016). The range of applications for imidacloprid result in potential for entry into aquatic systems through a variety of vectors, including spray drift, atmospheric deposition, soil erosion and runoff (CCME, 2007).

Imidacloprid was detected in all samples at 8 sites, which was the highest level of occurrence for all compounds (Table S1). In general, very high occurrences of detection were observed across the entire study area, presumably due to the broad range of applications of imidacloprid. There was roughly a 6000-fold range in measured concentrations from low ng/L to 10,400 ng/L (Table S1). Imidacloprid was particularly prevalent in southwestern Ontario along the Lake Erie shoreline (Lebo Drain and Sturgeon Creek, Fig. 1) and at Two Mile Creek in the Niagara Peninsula (Fig. 1). At Lebo Drain and Sturgeon Creek, roughly 75% of the samples contained imidacloprid at concentrations exceeding the CCME guideline value (230 ng/L). The watersheds of these water courses are characterized by high percentages of row crop agriculture; roughly 20% corn and 40% soybean for Lebo Drain and 26% corn and 33% soybean for Sturgeon Creek, Table S2). This area of southern Ontario is also home to the largest concentration of commercial greenhouses (representing 9% of the watershed, Table S2) in North America with roughly 1800 acres in vegetable production in 2012 (Ontario Greenhouse Vegetable Growers, 2012); cucumbers, peppers and tomatoes represent the majority of greenhouse crops in this area. In addition, 4.6% of the Sturgeon Creek watershed is dedicated to field tomato production. Two Mile Creek in the Niagara Region was the only other sampling station where concentrations of imidacloprid exceeded the CCME guideline (maximum value of 816 ng/L, Table S1). In contrast to watersheds in southwestern Ontario dominated by row crops, the Two Mile Creek watershed is represented by over 50% vineyards and orchards (Table S2). These observations indicated imidacloprid is preferred as an insecticide for a broad range of agricultural activities. These results contrasted with previous Canadian studies from 2000 to 2005 where imidacloprid was rarely detected.

Acetamiprid was one of the less ubiquitous compounds with only 6 sites exhibiting detection rates greater than 50% (Table S1, Fig. S3). As with imidacloprid, the highest concentrations and frequencies of detection were associated with sites in southwestern Ontario (Sturgeon Creek, Lebo Drain) and the Niagara Peninsula (Two Mile Creek, Prudhomme Creek and Four Mile Creek). In four samples acetamiprid was detected at concentrations greater than the imidacloprid CCME guideline value of 230 ng/L; these samples were associated with Two Mile Creek (1 sample), Lebo Drain (2 samples) and Sturgeon Creek (1 sample). Crops on which acetamiprid is routinely applied include pome fruits, leafy vegetables and ornamental plants and flowers; these crops are widely grown in both regions. In addition, acetamiprid is applied to grapes; the Niagara Region represents the majority of the 17,000 acres of grapes in production in Ontario with lesser production in the southwestern and eastern parts of the province (Grape Growers of Ontario, (2016)). Grapes comprise 35% of the value of Ontario commercial fruit crops. The Two Mile Creek and Four Mile Creek watersheds are characterized by roughly 26% orchard/33% vineyard and 15% orchard/28% vineyard, respectively (Table S2). Prudhomme Creek has watershed characteristics similar to those of Two Mile Creek and Four Mile Creek.

Thiacloprid exhibited the lowest levels of occurrence, distribution, and rate of detection (Table S1 and Fig. S4); Prudhomme Creek was the only site where thiacloprid was detected in greater than 50% of samples. The concentration of thiacloprid exceeded the imidacloprid CCME guideline value (230 ng/L) in only three samples; all of these occurrences were in Prudhomme Creek in the Niagara Region. The occurrence and distribution of thiacloprid is limited by its relatively narrow range of applications; in the case of its detection at sites in southwestern Ontario (Sturgeon Creek and Lebo Drain) and the Niagara Peninsula (Prudhomme Creek, Two Mile Creek and Four Mile Creek), application on pome fruits represents the most likely source, as registration in Canada is restricted to use on these crops. Although designated as the urban control site, thiacloprid was detected at 17% of samples collected at Indian Creek (Table S1, Fig. S4). Thiacloprid is not registered for domestic use in Canada; these detections may be the result of some limited agricultural activity in the watershed that includes orchards (Table S2).

Although clothianidin is registered for a fairly broad range of applications in Canada, it is commonly used as a seed treatment for canola and corn, and on grains and soybean. Of the twelve clothianidin commercial products registered for use in Canada, nine are for field crops; the remaining three are for turf grass, orchards and vegetables. The widespread prevalence of row crop agriculture in southern Ontario has resulted in clothianidin being ubiquitous as evidenced by its detection in over 80% of samples at 10 of the 15 sites in the study (Table S1, Table S2, Fig. S5). In addition to its frequent detection at sites in southwestern Ontario and the Niagara Region, clothianidin was prevalent in central and northern parts of southern Ontario where row crop agriculture is predominant (Fig. S5). For example, the Nissouri Creek (100% detection rate) watershed is roughly 40% corn production (Table S2). The concentration in only one sample in Two Mile Creek in the Niagara Peninsula exceeded the imidacloprid CCME guideline value of 230 ng/L; in general mean clothianidin concentrations were in the tens of ng/L (Table S1). Clothianidin is also the primary metabolite of thiamethoxam, although the current study provided no insights into the relationship of these compounds from a parent – breakdown product perspective.

Thiamethoxam is similar to clothianidin in that many registered commercial products are specifically for seed treatment; as a result there are similarities in the occurrence and distribution for both compounds (Figs. S5 and S6). However, thiamethoxam is registered for a broader range of applications compared to clothianidin, including control of house flies, ornamentals, greenhouse, and fruits and vegetables. As with clothianidin, typical mean concentrations for thiamethoxam are in the tens of ng/L range. Mean concentrations for thiamethoxam were highest at Twenty Mile Creek, Lebo Drain and the Sydenham River (Table S1); these mean concentrations also exceeded the 230 ng/L CCME guideline value.

The measured concentrations in the current study can be compared with those reported in other areas of North America and globally. Geometric means for average and maximum concentrations of all neonicotinoids in surface waters based on 29 studies carried out in 9 countries world-wide were 130 ng/L and 630 ng/L, respectively (Morrissey et al., 2015). For imidacloprid in the current

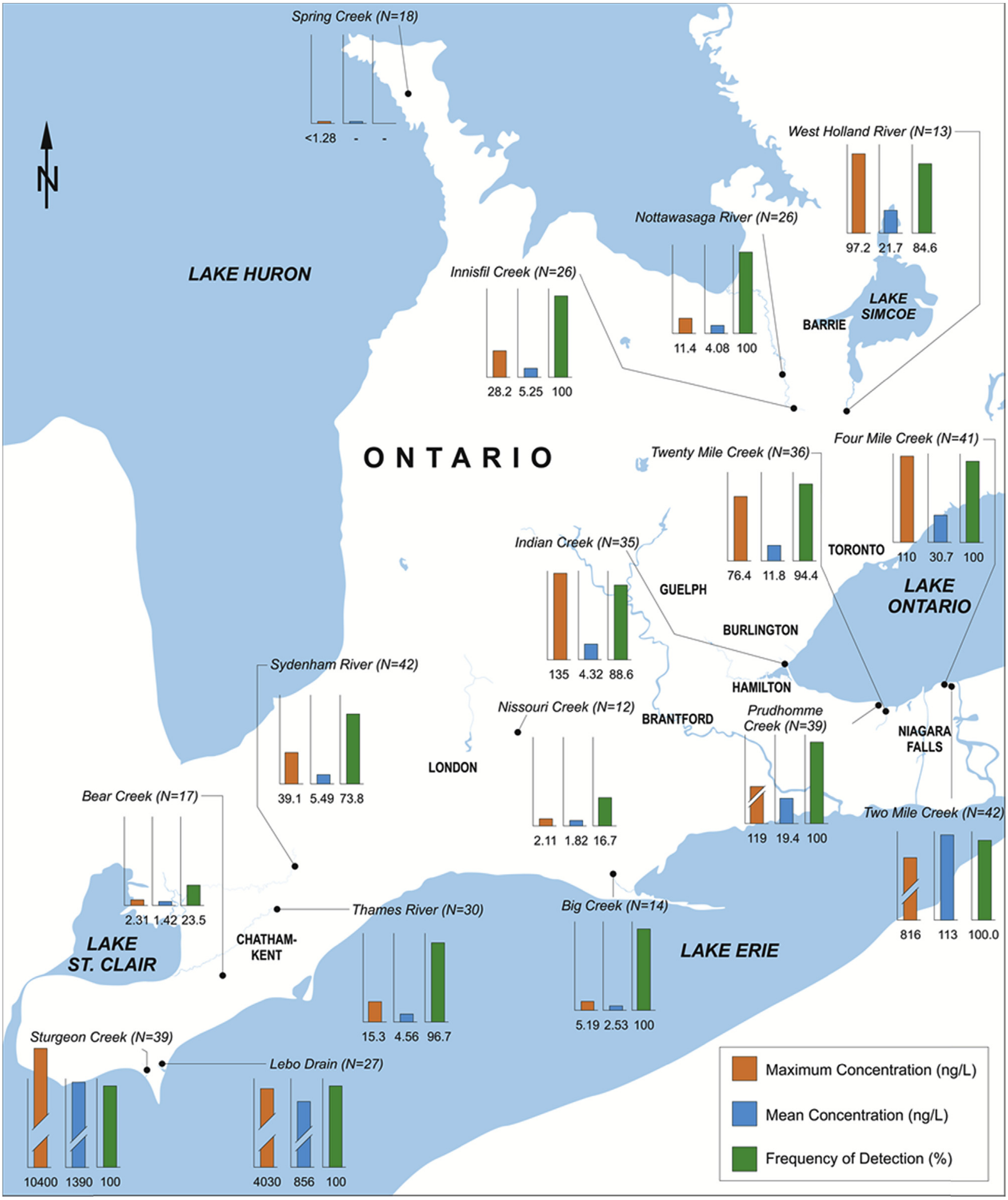

Fig. 1. Occurrence and distribution of imidacloprid in southwestern Ontario surface waters. Maximum and mean concentrations are expressed in ng/L while frequency of detection represents the percentages of samples in which imidacloprid was detected.

study, the mean average and maximum concentrations at the thirteen sites where the rate of detection was greater than 70% were 190 ng/L and 1210 ng/L, respectively. Morrissey et al. (2015) also presented a summary of ecological quality reference values for neonicotinoid insecticides, including the Canadian benchmark of 230 ng/L. The USEPA average (1050 ng/L) and maximum (35,000 ng/L) reference values are much higher than the Canadian CCME guideline value; however, the most recent benchmark for an average concentration adopted by the Netherlands (8.3 ng/L) is roughly 30-fold lower than the Canadian guideline. A study of neonicotinoids in the Province of Quebec (Canada) determined that 3.2%–48% of surface water samples collected from watercourses where corn and soybean were the predominant agricultural crops exceeded the Netherlands reference value of 8.3 ng/L (Quebec, 2014). Morrissey et al. (2015) proposed reference values for average and maximum concentrations of 35 ng/L and 200 ng/L, respectively; these values were developed in consideration of weighting and standardizing all neonicotinoid insecticides to imidacloprid.

3.2. Correlation of land use with occurrence and distribution of neonicotinoid insecticides in southern Ontario

The occurrence and distribution of neonicotinoid insecticides in southern Ontario surface waters were presumably primarily influenced by agricultural activities. To assess the importance of land use, we performed a Principal Components Analysis (PCA) to identify correlations between land use and neonicotinoids (Fig. 2). In general, results of the PCA corroborated our previous interpretations of the data; as expected thiamethoxam and clothianidin were positively correlated with row crops, particularly soybeans and corn, while imidacloprid and acetamiprid were strongly correlated with greenhouse activity, vegetables and other agriculture including vineyards and orchards. Thiacloprid was more associated with fruit production, as was expected given this

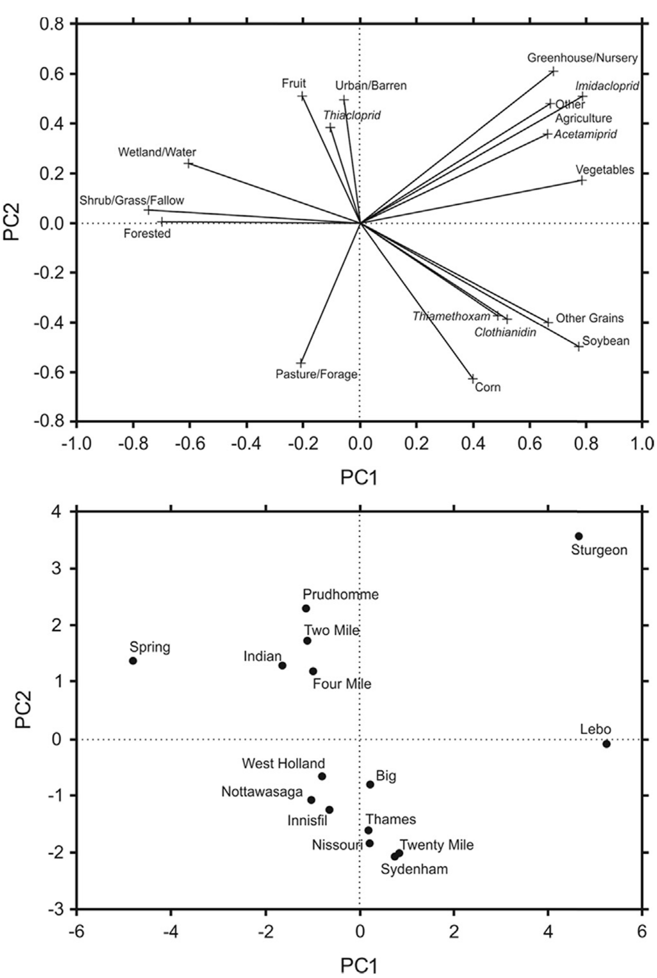

Fig. 2. Principal component analysis (PCA) of land use, crop type, and measured concentrations of neonicotinoid insecticides. The top panel shows the loadings of each factor and the bottom panel shows the distribution of stations in the ordination.

insecticide is commercially registrated in Canada for use on pome fruits (apples and pears). The most recent survey of pesticide use in Ontario (Farm and Food Care Ontario, 2016) reported that both corn and soybean acreage increased by over 20%, while associated pesticide usage increased by 38% for corn and 32% for soybeans; as a result, use of neonicotinoid insecticides for these two crops can be expected to continue to be robust. In their national-scale reconnaissance of neonicotinoids in the USA, Hladik and Kolpin (2015) found a positive statistical relationship between row crops and both clothianidin and thiamethoxam, while imidacloprid exhibited a positive relation to urban land-use. In terms of the co-occurrence of clothiainidin and thiamethoxam, Hladik and Kolpin (2015) also identified transformation of thiamethoxam to clothianidin as a potential factor.

Sampling sites in the PCA were generally grouped according to geography, and correspondingly, land use (Fig. 2). One grouping contained sites in the Niagara Peninsula (Prudhomme Creek, Two Mile Creek, Four Mile Creek, Fig. 2) and the urban control site (Indian Creek), while the other contained the sites in southwestern and southcentral Ontario. Twenty Mile Creek was also in the latter grouping as a result of this watershed representing primarily row crop agricultural activities, in contrast to the other sites in this area that exhibit a diversity of agricultural activities that include orchards and vineyards (Table S2). Interestingly, the Lebo Drain and Sturgeon Creek sites are significantly separated in the PCA; these two watercourses are adjacent to each other and routinely monitored for water quality. For both Lebo Drain and Sturgeon Creek, intensive horticultural activities, not exclusively limited to greenhouses, have contributed to elevated nutrient levels at the mouths of both watercourses (OMOE, 2012). The relatively greater influence of greenhouse activity in the Sturgeon Creek watershed (roughly 3-fold greater on a percentage basis, Table S2), and differences in maximum and mean neonicotinoid concentrations due to differences in the physical characteristics of these watersheds, is apparent.

3.3. Seasonal trends in occurrence and distribution of neonicotinoid insecticides in southern Ontario

Multi-year monitoring of neonicotinoid insecticides during field season (April to December) allows for assessment of seasonal trends in occurrence and distribution. In general, we observed two types of distributions dependent on insecticide and/or crop type. We selected Four Mile Creek and Prudhomme Creek, and Lebo Drain and Sturgeon Creek as representative examples of these different seasonal distributions. In all cases, we observed high rates of detection and high concentrations of neonicotinoid insecticides in spring in concert with snow melt, spring rains and subsequent crop planting; this "spring flush" phenomenon has been observed in other studies (e.g., Hladik et al., 2014).

In the case of Four Mile Creek and Prudhomme Creek, there was no apparent seasonal trend in the occurrence of imidacloprid, with high rates of detection throughout the spring/summer/fall time periods (Fig. 3). We attribute the lack of a seasonal trend at the Niagara sites to a wide range of agricultural activities potentially requiring multiple applications across a broader period of time and throughout the growing season. For example, imidacloprid can be used for preventative purposes in mid-summer; in addition, this compound is applied using a variety of techniques including soil treatment and foliar spray application that in turn increases the number of potential vectors for entry into watercourses. A similar temporal distribution was observed at the Four Mile Creek and Prudhomme Creek sites for acetamiprid (data not shown). In contrast, the occurrence of imidacloprid at Sturgeon Creek and Lebo Drain exhibited a bimodal distribution with maxima in late

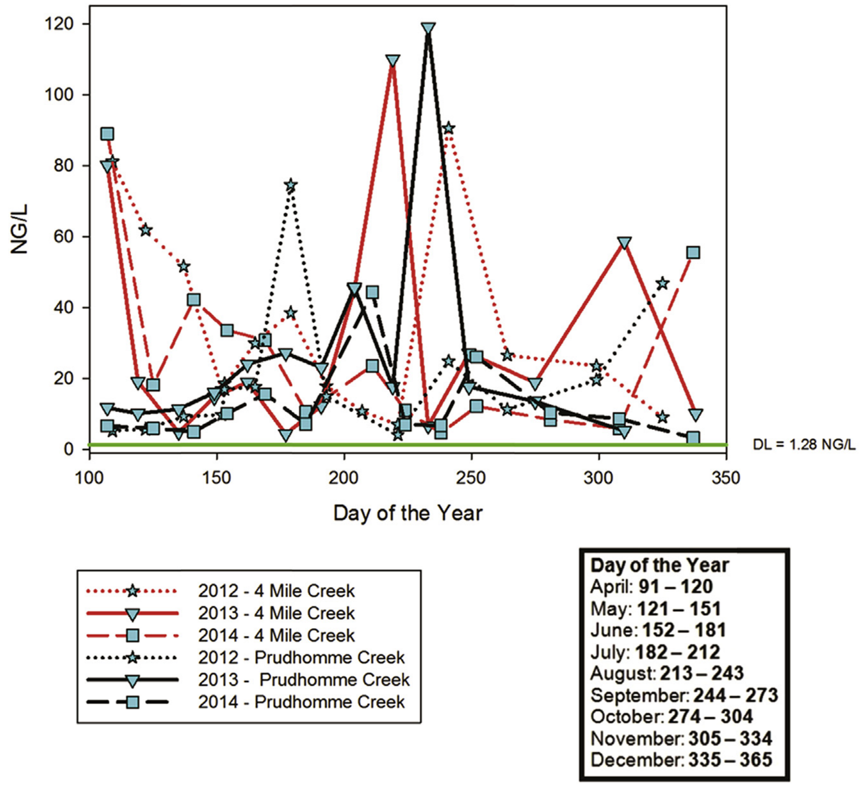

Fig. 3. Occurrence of imidacloprid at Four Mile Creek and Prudhomme Creek in the Niagara Region of southern Ontario resulting from sampling conducted from April to December 2012–2014.

spring and late summer/early fall (Fig. S7). We attribute this observation to greenhouse and/or vegetable applications; these watercourses have the highest level of greenhouse activity of all watersheds surveyed (Table S2, Fig. 2). Typical application periods for insecticides on row crops include spring and fall; however, it has also been reported that imidacloprid, clothianidin and thiamethoxam can be detected at significant concentrations (>100 ng/L) five-to-six months after use as seed treatments (Mineau and Palmer, 2013). Therefore, the bimodal distributions we observed at some sites could be the result of seasonal application, release of residues several months after application, or a combination of both circumstances.

Seasonal patterns for detection of neonicotinoid insecticides applied primarily for protection of row crops were more definitive; the occurrence of thiamethoxam at Four Mile Creek and Prudhomme Creek in the Niagara Region is shown in Fig. S8. The distribution of occurrence was bimodal and exhibited maximum concentrations in the late spring and fall time periods. We observed similar distributions for both thiamethoxam and clothianidin at other sampling sites, including Lebo Drain and Sturgeon Creek (data not shown). However, in the case of clothianidin at Four Mile Creek and Prudhomme Creek, we observed a unimodal distribution corresponding to maximum concentrations primarily in the spring (Fig. S9). We are unsure of the reasons for the lack of detections of clothianidin at the Niagara Region sites later in the year, compared to other areas of southern Ontario, but presume that preventative and/or curative applications in summer and fall were generally not required using this compound in these areas over the period of the study. Over the course of the three-year sampling program, we observed a broader time period when soybeans were being planted (e.g., into late-June), compared to corn which is not planted after May in Ontario.

3.4. Correlation of precipitation with occurrence and distribution of neonicotinoid insecticides in southern Ontario

The high water solubility of some neonicotinoid insecticides, including imidacloprid, was a primary impetus for investigating the relationship between precipitation and occurrence, as runoff is an important vector for entry of neonicotinoid insecticides into the aquatic environment. In their national-scale reconnaissance of neonicotinoids in the USA, Hladik and Kolpin (2015) identified precipitation as an important driver of neonicotinoid runoff to watercourses. Sampling in conjunction with rain events has been identified as a crucial factor in interpreting both peak and mean concentrations of neonicotinoids in surface waters, and the associated exposure of aquatic species (Morrissey et al., 2015). The occurrence and fate in surface waters are influenced by light, pH, temperature, formulation and microbial processes (Anderson et al., 2015). Three watercourses (Sydenham River, Four Mile Creek, Twenty Mile Creek, Fig. S1) were selected for assessment of the relationship among occurrence and rainfall events and water flow(stream discharge); supplemental monitoring of these sites resulted in availability of precipitation data of sufficient frequency and resolution to enable statistical analysis. However, it should be noted that precipitation measurements were not conducted at the exact location of surface water sampling.

A statistical analysis of the correlations among neonicotinoid insecticides and stream discharge, precipitation on the day preceding sampling, and stream discharge on the day of sampling was performed (Table 1). In addition, the correlations between the individual compounds were calculated (Table S3). There were no correlations between neonicotinoids and precipitation on the day of sampling; all significant correlations were associated with stream discharge and/or precipitation on the day preceding

Table 1
Non-parametric correlation coefficients (Kendall's τ) of concentrations of selected neonicotinoid insecticides with precipitation on day of sampling, precipitation on the day preceding sampling, and stream discharge on day of sampling measured at three locations (2012–14). Correlation coefficients in *italics* designate p-values <0.10 while **bold** designates p-values <0.05.

	Stream discharge (m^3/s)	Precipitation - sampling day (mm)	Precipitation - day preceding sampling (mm)
Imidacloprid			
Sydenham River	−0.085	−0.045	0.177
Four Mile Creek	0.136	−0.048	*0.197*
Twenty Mile Creek	**0.346**	0.080	0.149
Clothianidin			
Sydenham River	**0.345**	−0.003	0.002
Four Mile Creek	0.105	0.120	*0.217*
Twenty Mile Creek	**0.255**	**0.251**	0.143
Thiamethoxam			
Sydenham River	0.022	−0.063	0.042
Four Mile Creek	**0.219**	0.077	**0.341**
Twenty Mile Creek	**0.264**	**0.280**	0.195
Acetamiprid			
Sydenham River	−0.025	0.145	0.133
Four Mile Creek	0.150	0.121	0.167
Twenty Mile Creek	0.150	0.045	0.220
Thiacloprid			
Sydenham River	0.168	−0.201	0.083
Four Mile Creek	0.096	0.086	0.167
Twenty Mile Creek	0.079	−0.108	**0.337**

sampling (Table S3). In terms of correlations between individual neonicotinoids, thiamethoxam and clothianidin were correlated in all three watercourses due to similarities in crop types to which they are applied (Table S3).

For the Sydenham River, two significant positive correlations were observed; precipitation on the day preceding sampling for imidacloprid ($p < 0.10$) and stream discharge for clothianidin ($p < 0.10$). As shown by the hydrograph for the Sydenham River (Fig. S10), we anticipated difficulty in attributing occurrence of neonicotinoids with precipitation events due to the fact this watercourse is a major tributary characterized by high flow volumes and discharge; as a result any runoff from the watershed could be rapidly diluted. For the Sydenham River, there was no correlation between precipitation and stream discharge.

In the case of Four Mile Creek, precipitation on the day of sampling and stream discharge were significantly correlated; as a result precipitation has a significant impact on stream discharge throughout the year (Fig. S11). Clothianidin was positively correlated ($p < 0.10$) with precipitation the day preceding sampling while thiamethoxam was more strongly correlated ($p < 0.05$) for both precipitation the day preceding sampling and stream discharge, indicating that precipitation events and the associated runoff are significant contributors of loadings of neonicotinoids primarily associated with row crop agriculture to watercourses (Table 1). For Twenty Mile Creek, precipitation preceding the day of sampling and stream discharge were significantly correlated (Fig. 4). Compared to Four Mile Creek, Twenty Mile Creek exhibited high stream discharges in the late winter – early spring time period. Imidacloprid ($p < 0.05$) and clothianidin ($p < 0.10$) were both correlated with stream discharge for this watercourse, while thiamethoxam ($p < 0.05$) was correlated with precipitation the day preceding sampling (Table 1). The observations of neonicotinoid occurrence in

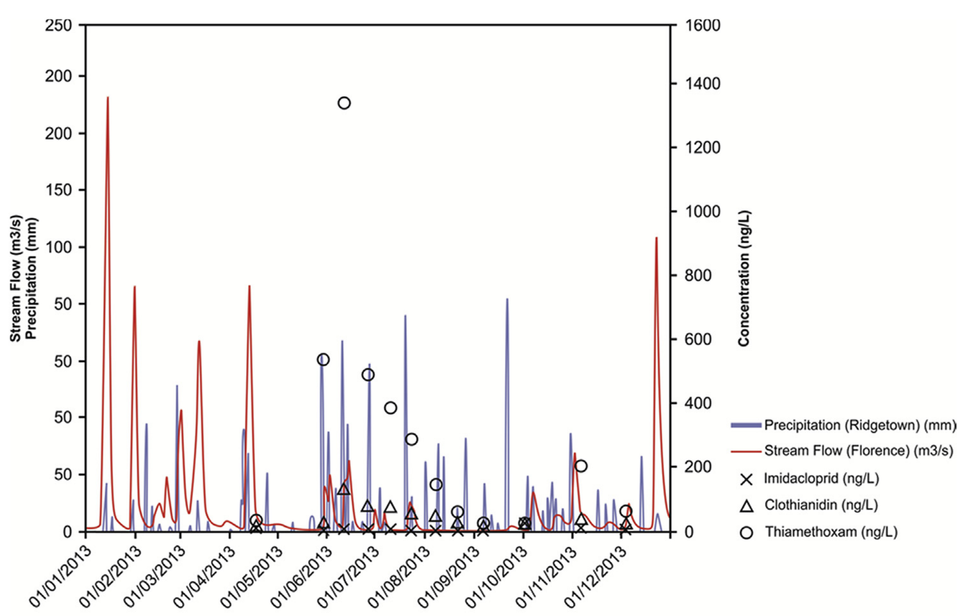

Fig. 4. Hydrograph showing stream flow (m^3/s), precipitation (mm) and neonicotinoid insecticide concentrations (ng/L) for sampling in 2013 in Twenty Mile Creek.

the Niagara Region show that in cases where precipitation and stream discharge are correlated, sampling associated with precipitation events is important for assessing the influence of agricultural activities on smaller watercourses in that runoff can exhibit relatively high concentrations and represent significant loadings, compared to base flow conditions. In the case of thiamethoxam in Twenty Mile Creek, the hydrograph also showed the influence of precipitation events in the late spring and summer months that can result in release of neonicotinoids to watercourses (Fig. 4).

Neonicotinoids were rarely detected in precipitation at Bear Creek in 2013; most detections were during the period of 14–31 May 2013. While detections of imidacloprid, thiacloprid and acetamiprid were within a factor of two of the method detection limit, concentrations in precipitation of thiamethoxam and clothianidin on May 14th, 2013 were 114 ng/L and 120 ng/L, respectively. We speculate these detections may have been the result of drift of dust generated during application on row crops, or planting of treated seeds during the spring planting period, given that the Bear Creek site is in proximity to the Lebo Drain and Sturgeon Creek stations, both of which are characterized by greater than 60% row crop agriculture.

4. Conclusions

The most widely used of the neonicotinoid insecticides; imidacloprid, thiamethoxam and clothianidin, were detected in over 90% of samples from over half of the sites surveyed during the three years of the study (2012–2014). Based on usage information for the Province of Ontario, the ubiquity of these compounds was not unexpected, and our data corroborate findings of studies conducted elsewhere in Canada and the United States. In the case of imidacloprid, the broad range of registered uses combined with multiple methods of application resulted in a high frequency of detections in surface waters across southern Ontario; however, there was a roughly 6000-fold range in concentrations detected. In addition to high frequencies of detection of neonicotinoids in spring samples, at some sites in the Niagara Peninsula area of southern Ontario imidacloprid was detected over the breadth of the spring – summer – fall timeframe, which indicated multiple applications during the planting and growing seasons. In one area of southwestern Ontario, three quarters of the samples exceeded the Canadian guideline value for imidacloprid (230 ng/L) indicating this compound is environmentally relevant and should continue to be the focus of further research and monitoring activities.

As expected, the occurrence and distribution of thiamethoxam and clothianidin were also correlated with row crop agriculture resulting from their wide use as seed treatments for canola, corn, grains and soybean. Seasonal patterns of detection for these two compounds were bimodal in nature, with maximum concentrations observed in late spring and fall. Recent information from the Province of Ontario indicates continued increases in acreage devoted to row crop agriculture which could significantly influence use of neonicotinoids. The results of the current study in southern Ontario also emphasize the importance of greenhouse activity in influencing the occurrence and distribution of neonicotinoids in surface waters. In terms of the impact of precipitation and watercourse characteristics on the occurrence of neonicotinoid insecticides, the results of our study were more definitive in cases where precipitation and discharge were linked, i.e., for smaller watercourses; in these cases precipitation events and subsequent runoff and increased discharge resulted in higher concentrations and loadings.

The results of our study emphasize the need for targeted event-based sampling to determine maximum concentrations and their duration in surface waters, and the requirement for ecotoxicological studies to investigate potential acute and chronic effects on a range of aquatic biota.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.11.036.

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