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Research Paper

First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA

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Environmental context. Neonicotinoids are under increased scrutiny because they have been implicated in pollinator declines and, more recently, as potential aquatic toxicants. Nevertheless, there is currently little information on concentrations of multiple neonicotinoids in surface water. This paper presents a summary of concentrations of six neonicotinoids in streams from across the United States in both urban and agricultural areas. These environmental data are important in determining the potential risk of neonicotinoids to non-target aquatic and terrestrial organisms.

Abstract. To better understand the fate and transport of neonicotinoid insecticides, water samples were collected from streams across the United States. In a nationwide study, at least one neonicotinoid was detected in 53 % of the samples collected, with imidacloprid detected most frequently (37 %), followed by clothianidin (24 %), thiamethoxam (21 %), dinotefuran (13 %), acetamiprid (3 %) and thiacloprid (0 %). Clothianidin and thiamethoxam concentrations were positively related to the percentage of the land use in cultivated crop production and imidacloprid concentrations were positively related to the percentage of urban area within the basin. Additional sampling was also conducted in targeted research areas to complement these national-scale results, including determining: (1) neonicotinoid concentrations during elevated flow conditions in an intensely agricultural region; (2) temporal patterns of neonicotinoids in heavily urbanised basins; (3) neonicotinoid concentrations in agricultural basins in a nationally important ecosystem; and (4) in-stream transport of neonicotinoids near a wastewater treatment plant. Across all study areas, at least one neonicotinoid was detected in 63 % of the 48 streams sampled.

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Introduction

There is increasing concern about neonicotinoid insecticides not only to pollinators[1] but also to other organisms such as those residing in aquatic environments.[2] Elevated surface-water concentrations of imidacloprid have been correlated with direct effects on invertebrates[3] and indirect effects on insectivorous birds[4] and some fish.[5] Recent research has focussed on not only acute toxicity of neonicotinoids but also chronic toxicity, especially to aquatic invertebrates that may be exposed to neonicotinoids by water.[2]

Neonicotinoid use has continued to increase both in the United States[6,7] and worldwide.[8,9] They are applied in both agricultural (foliar sprays, in-furrow treatments and seed coatings) and urban (lawn and garden foliar sprays, granular, tree injections; companion animal flea treatment) settings. Neonicotinoids are highly water-soluble (octanol–water partition coefficients, log K_{ow}, range from −0.55 to 1.26)[10] with fairly long soil degradation half-lives (DT_{50} 3 to 545 days),[10] making them both mobile and persistent with the potential for offsite transport to adjacent water bodies.[11,12]

The lack of surface-water data is considered an important knowledge gap for neonicotinoids.[13] This information is needed to accurately assess potential environmental effects from neonicotinoid exposures from stream concentrations. Current surface-water data exist primarily for imidacloprid, the mostly widely use neonicotinoid globally.[8] More recent studies, however, have documented mixtures of neonicotinoids in wetlands,[14–16] groundwater[12] and surface water.[11,17–19] An example of such research documented that neonicotinoid mixtures were prevalent in streams in the Midwestern US, even in the largest systems (i.e. Missouri and Mississippi Rivers), with substantial temporal pulses of neonicotinoids in streams following rainfall events during crop planting likely attributed to seed treatment applications.[11]

The current study provides the first national-scale assessment of neonicotinoids in USA streams that has been conducted to date. In addition, targeted research studies were conducted to complement these national-scale results to enhance our understanding of the contributions of both agricultural and urban neonicotinoid use to stream concentrations. Such national- and regional-scale data sets provide important geographic and temporal data that can be used to provide important baseline concentration information for determining potential environmental effects from exposure to stream neonicotinoid concentrations.

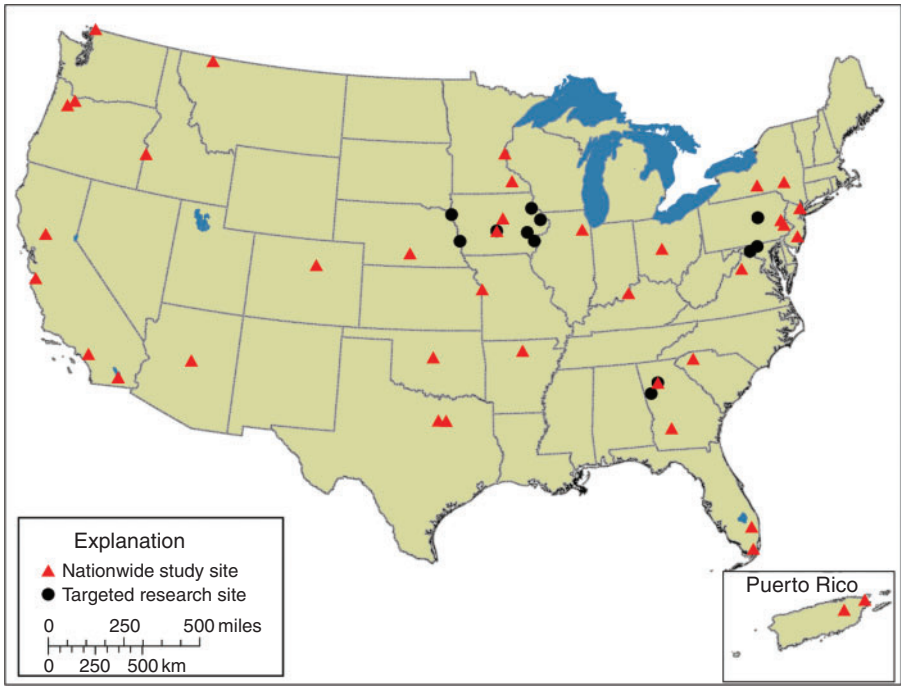

Fig. 1. Map showing sample locations for the nationwide study (2012–14) and targeted research studies (2011–14).

Table 1. Site information, neonicotinoid concentrations and instantaneous loads for samples collected from Iowa stream and rivers after heavy rains and flooding in 2014

Samples collected in 2013 for these sites are also included

Site	USGS site ID	Drainage area (km^2)	Date	Discharge ($m^3 s^{-1}$)	Clothianidin (ng L^{-1})	Imidacloprid (ng L^{-1})	Thiamethoxam (ng L^{-1})	Total neonicotinoid load (g s^{-1})
Missouri River at Omaha, NE	06610000	836 000	19-Jun-13	1150	18	4.4	11	0.038
			18-Jun-14	2080	25	11	18	0.11
			–					
North Fork Maquoketa River near Fulton, IA	05418400	1310	24-Jun-13	28	74	4.8	40	0.003
			19-Jun-14	77	98	11	40	0.011
			–					
Old Mans Creek near Iowa City, IA	05455100	521	26-Jun-13	36	84	23	26	0.005
			01-Jul-14	157	68	25	20	0.018
			–					
Iowa River at Wapello, IA	05465500	32 400	01-Jul-13	1420	62	15	30	0.15
			03-Jul-14	3030	53	19	20	0.28
			–					
Big Sioux River at Sioux City, IA	06485950	24 400	20-Jun-14	1821	77	20	38	0.25
			–					
Turkey River at Garber, IA	05412500	4000	20-Jun-14	708	132	26	73	0.16

Experimental

Sampling

National study

To obtain the first nationwide data set on neonicotinoids, 38 streams across 24 US states and Puerto Rico were sampled one time for neonicotinoids between November 2012 and June 2014 (Fig. 1; Table S1). These samples were collected as part of larger project to assess the human and ecological health risks associated with exposure to complex chemical mixtures.[20] Thirty-four of the streams were specifically selected owing to a wide range of contaminant sources within their watersheds (e.g. agricultural and urban sources). In addition, four streams were selected as biological reference sites because they are considered to have fish and aquatic macroinvertebrate communities that have been minimally disturbed by human development. The sampled watersheds range in size from 12 to 16 200 km^2 (median = 170 km^2).[20] All water samples were collected from the centroid of flow. Additional information on the site characteristics can be found in Table S1.

Streams during elevated hydrologic conditions: Iowa, 2014

To determine neonicotinoid concentrations in streams during elevated hydrologic conditions in an intensely agricultural region, grab samples from six sites in Iowa (Table 1; Fig. 2a)

were collected from the centroid of flow following heavy rainfall and after most row crops (corn and soybeans) had been planted in these watersheds. Four out of the six sites were above flood stage and all samples were collected near the crest of the hydrograph. The drainage area for the basins sampled ranged from 521 to 836 000 km^2.

Temporal patterns in urban streams in the Southeast

Two urban-affected streams (Sope Creek, 80 km^2, 39 % urban; Chattahoochee River, 6300 km^2, 18 % urban) were frequently sampled (67 total samples) in Georgia to better understand temporal variations in stream neonicotinoid concentrations derived from urban sources (Fig. 2b). All water samples from these sites were collected through depth and width-integrated composites.[19,21] Samples were collected from Sope Creek every 2 weeks on a set schedule for 2 years starting in October 2011 (48 samples collected). Samples were collected from the Chattahoochee River on an alternating once or twice per month set schedule (19 samples collected). No samples from these two sites were collected in relation to any specific flow conditions.

Streams in a nationally important ecosystem: Chesapeake Bay

As part of a larger study to determine if chemical exposure is contributing to fish health issues being observed in the Chesapeake Bay watershed,[22–24] water samples were collected from three sites in this watershed (Fig. 2c): six samples from Antietam Creek (725 km^2, 25 % cultivated crops, 10 % urban); seven samples from Big Pipe Creek (270 km^2, 41 % cultivated crops, 2 % urban); and four samples from Chillisquaque Creek, (290 km^2, 32 % cultivated crops, 2 % urban). Automatic samplers were used to collect water.

In-stream transport: Fourmile Creek, Iowa

To better understand the contributions of neonicotinoids to streams from wastewater treatment plant (WWTP) discharge and their in-stream fate, a 4.8-km reach of Fourmile Creek, located near Ankeny, Iowa, was investigated (Fig. 2a). The study reach extends from 1.9 km above the WWTP outfall (46×10^6 L day^{-1}; activated sludge treatment) to 2.9 km downstream of the WWTP outfall. In addition to a WWTP effluent sample, stream samples were collected at −1.9, −1.6, −0.08, −0.05, 0.05, 0.33 and 2.9-km distance in relation to WWTP outfall. Samples were collected in a Lagrangian approach, in which the same approximate parcel of water was tracked as it moved downstream, as was conducted previously in this study reach.[25–27] All samples were collected at the centroid of flow.

The proportion of stream flow derived from WWTP effluent below the outfall varies depending on antecedent moisture conditions. The first sampling of this study reach occurred from 5 to 6 December 2012 during a time of prolonged drought conditions. Thus, the stream flow below the outfall was 0.17 m^3 s^{-1}, with 99 % of this flow derived from effluent. The second sampling occurred on 20 June 2013 during more normal early-summer flow conditions, with the stream flow below the outfall at 1.7 m^3 s^{-1}, with 11 % of this flow derived from effluent.

Analytical method

All samples were placed in 1-L amber glass bottles and chilled at 4 °C until extraction. The six neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid and thiamethoxam) were measured in the water samples using a previously published method.[19] Samples were filtered with a 0.7-mm glass-fibre filter (Whatman, Piscataway, NJ), spiked with a surrogate (imidacloprid-d_4; Cambridge Isotope, Andover, MA), and passed through an Oasis HLB solid-phase

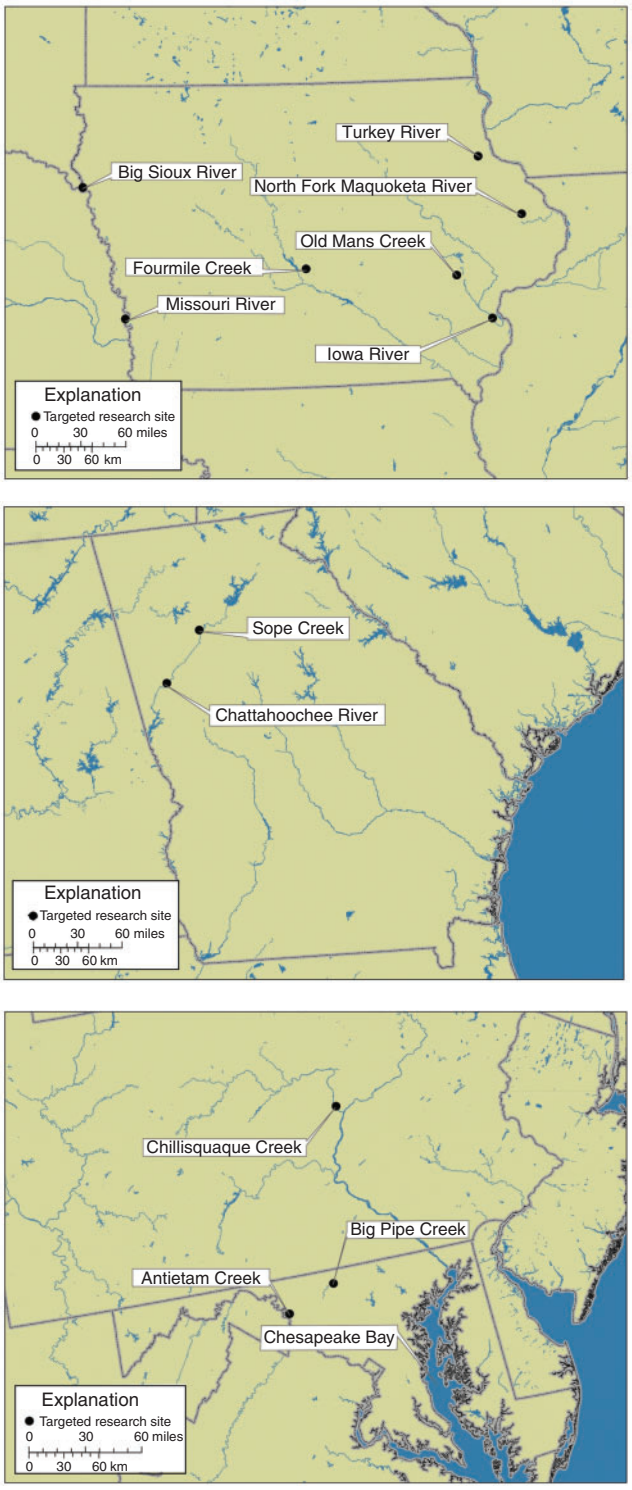

Fig. 2. Sites sampled as part of targeted research studies: elevated hydrologic conditions and in-stream transport in an intense agricultural region in Iowa (a); urban streams in the south-eastern USA (b); and Chesapeake Bay (c).

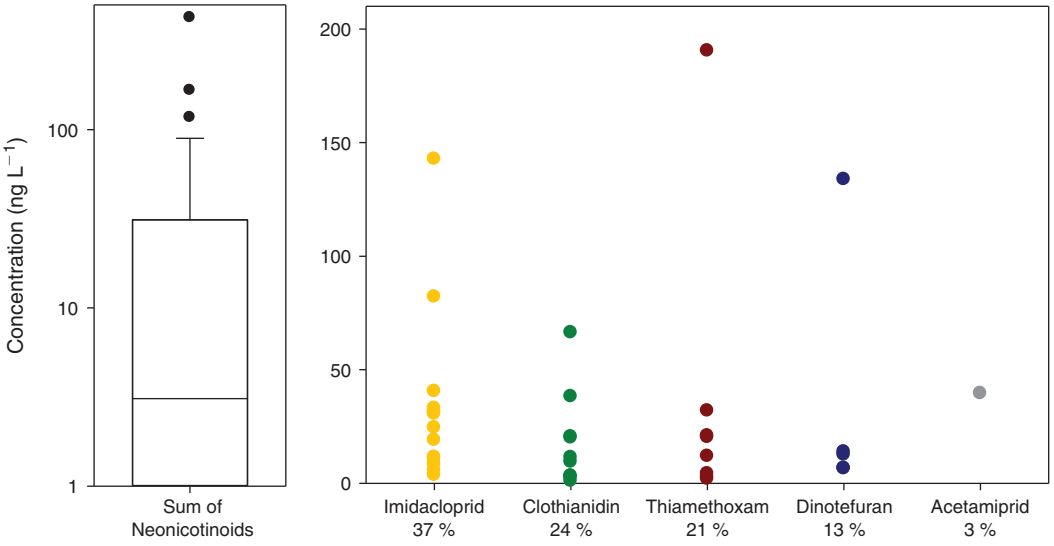

Fig. 3. Box plot of total neonicotinoids detected at 38 sites in a nationwide study from 2012 to 2014. Scatterplots show the range of individual neonicotinoid concentrations for the five compounds detected (out of six measured); overall detection frequency is listed underneath the compound names.

extraction (SPE) cartridge (6 mL, 500 mg; Waters Corporation, Milford, MA). The cartridge was eluted with 10 mL of 50 : 50 dichloromethane : acetone, reduced under nitrogen and an internal standard, $^{13}C_3$-caffeine, was then added. Extracts were analysed on an Agilent 1260 bio-inert liquid chromatograph (LC) coupled to an Agilent 6430 tandem mass spectrometer (MS-MS) (Santa Clara, CA). The theoretical level of detection (LOD) was 2 ng L^{-1} and the method detection limits (MDL) ranged from 3.6 to 6.2 ng L^{-1}.[19]

Neonicotinoid concentrations were validated against a set of quality control parameters including: field blanks (9), replicate samples (17), matrix spikes (8) and surrogate recovery. No compounds were detected in any of the blanks, field replicates had relative percentage differences (RPD) between the regular and replicate sample of <25 %. Matrix spike recoveries ranged from 70 to 102 %. Recovery of the surrogate (imidacloprid-d_4) ranged from 70 to 120 % for all samples; data presented here were not recovery-corrected.

Results and discussion

A total of 149 stream samples were collected and analysed for six neonicotinoids in one national-scale and four complementary research studies. A summary of the results of these efforts follows.

National study

Five of the six neonicotinoids measured were detected in this first ever national-scale study (Table S1). Although these samples represent only a single snapshot in time for the 38 sites sampled, they do represent spatial variations in stream neonicotinoid concentrations across the USA (Fig. 1). At least one neonicotinoid was detected in 53 % of the 38 sites sampled. Imidacloprid was the most frequently detected neonicotinoid (37 %, maximum concentration 140 ng L^{-1}), followed by clothianidin (24 %, 66 ng L^{-1}), thiamethoxam (21 %, 190 ng L^{-1}), dinotefuran (13 %, 130 ng L^{-1}) and acetamiprid (3 %, 40 ng L^{-1}) (Fig. 3, Table S1). Thiacloprid was not detected in any of the samples collected. Of the 37 detectable concentrations of individual neonicotinoids, 92 % were <100 ng L^{-1} with the median

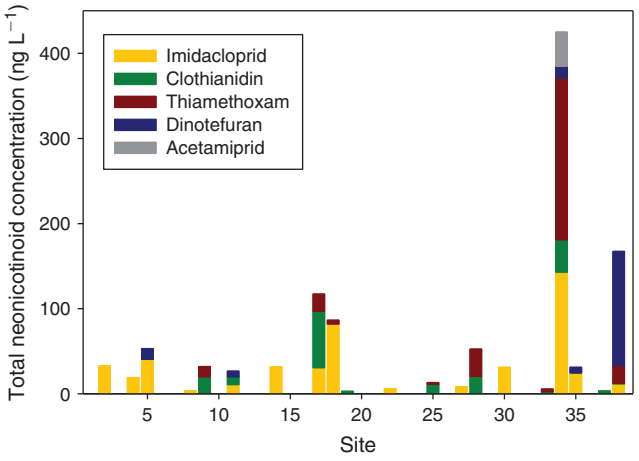

Fig. 4. Concentrations of neonicotinoid insecticides at 38 individual sites collected as part nationwide study from 2012 to 2014. The site number corresponds to the full names given in Table S1.

detected concentration of 19 ng L^{-1}. When summed, the highest total neonicotinoid concentration for a given sample was 450 ng L^{-1} (Fig. 4). Mixtures of multiple neonicotinoids in a single sample were common; two or more were detected in 26 % of the samples, three or more were detected in 11 % of the samples, and one sample (3 %) had five neonicotinoids detected (Table S1).

To provide a better understanding of neonicotinoid sources, an examination of the relationship between concentration and land-use (Table S1) was conducted (Table S2). This analysis determined a significant, positive relation (using Spearman's rank correlation) to cultivated crops for clothianidin ($\rho = 0.465$, $P = 0.003$) and thiamethoxam ($\rho = 0.472$, $P = 0.003$) and a positive relation to urban land-use for imidacloprid ($\rho = 0.474$, $P = 0.003$). These were expected relations to land-use based on the primary use of these neonicotinoids. In addition, a significant positive relation was observed between the two principal agriculturally used neonicotinoids, clothianidin and thiamethoxam ($\rho = 0.668$, $P < 0.001$). Their co-occurrence can at least partially be explained by the fact that both neonicotinoids

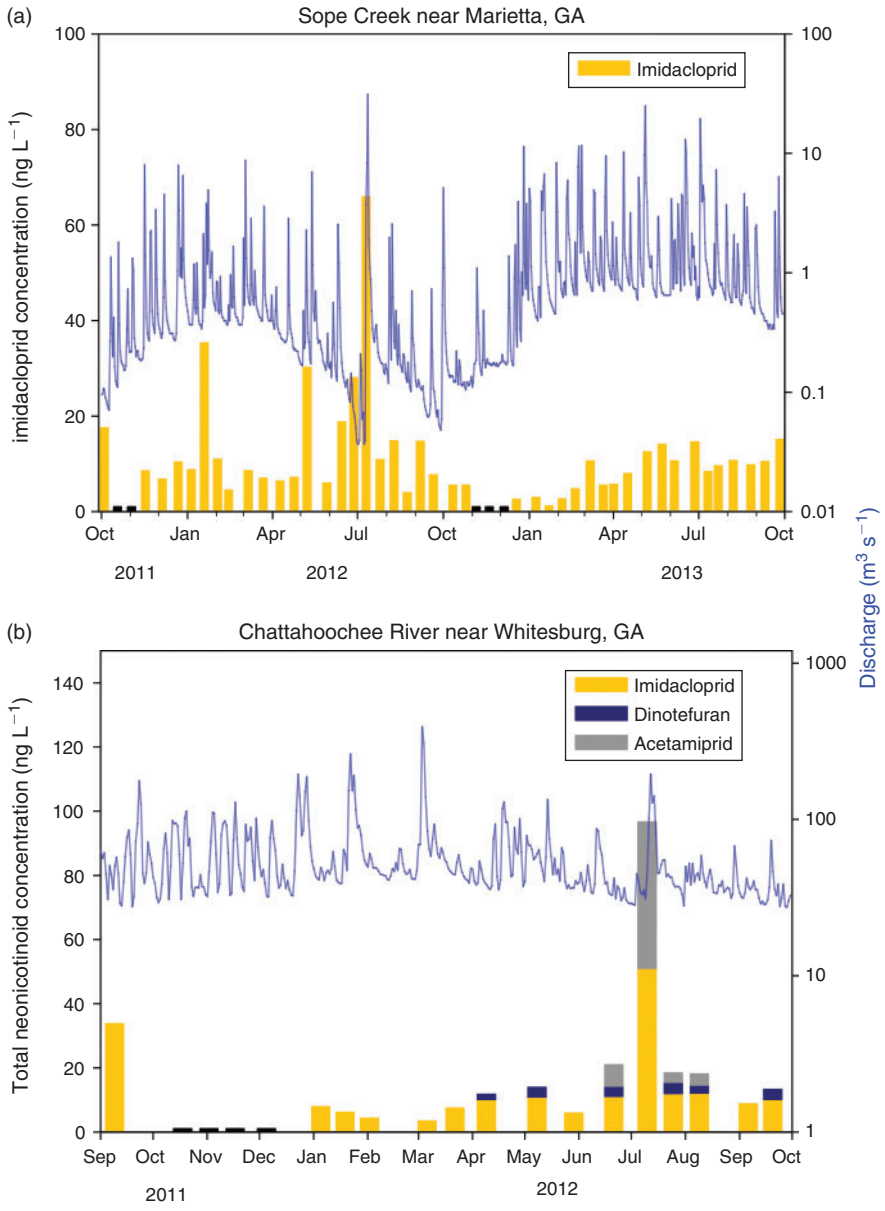

Fig. 5. Concentrations of imidacloprid and the corresponding stream discharge from October 2011 to October 2013 for Sope Creek (a). Concentrations of imidacloprid, dinotefuran and acetamiprid along with the corresponding stream discharge from September 2011 to September 2012 for Chattahoochee River (b). Black bars represent samples where no neonicotinoids were detected.

are primarily used on cultivated crops and that clothianidin is also a transformation product of thiamethoxam.[7,28] There were no significant relations among the other neonicotinoids under investigation (Table S2).

Streams during elevated flow conditions: Iowa, 2014

Because of the generally wet conditions across much of Iowa during the spring and early summer of 2014, a set of water samples from six sites were collected (Fig. 2a) to determine the effect such elevated flow conditions would have on stream neonicotinoid concentrations in an intensely agricultural region. Neonicotinoids were present in all six samples collected (Table 1). These 2014 results were similar to those collected during this same general time period in 2013[11]; median individual neonicotinoid concentration was 23 ng L^{-1} in 2014 and 25 ng L^{-1} in 2013 for sites with samples collected during both years. Although stream concentrations were similar between 2013 and 2014, the wet conditions in 2014 did cause substantially higher stream flows compared with 2013, which translated to higher instantaneous neonicotinoid loads (Table 1); thus, neonicotinoid loads were two to four times higher in 2014 than in 2013. These results confirm that precipitation is an important driver of neonicotinoid transport to streams following periods of use; even when such precipitation is heavy enough to cause substantial stream flooding, the neonicotinoid concentrations were not reduced.

Temporal patterns in urban streams in the Southeast

For this research component, stream samples were collected from two urban-affected streams (Fig. 2b): Sope Creek and the Chattahoochee River on a fixed sampling schedule. Not surprisingly, imidacloprid was the dominant neonicotinoid present

in these urban-affected streams, being detected in 87 % of 67 samples collected. Dinotefuran (10 %) and acetamiprid (7 %) were detected sporadically and were found along with imidacloprid (Table S3).

Imidacloprid was present in almost every sample (90 %) collected from the heavily urbanised Sope Creek (39 % urban) (Fig. 5a). Unlike what has been observed in agricultural streams in the Midwest,[11] there was no significant relation between imidacloprid concentrations and stream flow ($\rho = 0.21$, $P = 0.17$). This may have been at least partially due to the lack of a distinct use period for imidacloprid in such an urban-affected basin and the lack of a distinct growing season in the warmer climate of the south-eastern United States. In addition, the frequent events (e.g. frequent flushing of the system) observed in Sope Creek (Fig. 5a) and the warmer climate (possibly leading to increased degradation rates) may also have contributed to differences when comparing these urban-affected results with agricultural areas of the Midwest.

For the Chattahoochee River, which includes the Sope Creek drainage, one or more neonicotinoids were detected in 79 % of samples, two or more in 37 %, and three or more in 16 % of the samples collected. Imidacloprid was the most frequently detected neonicotinoid (79 %), followed by dinotefuran (32 %) and acetamiprid (5 %; Table S3). The more varied neonicotinoid detections in the Chattahoochee River compared with Sope Creek were likely a reflection of the more diverse land-use because the larger Chattahoochee watershed is 18 % urban and includes 9 % pasture (no cultivated crops, however). As with Sope Creek, there were frequent runoff events (Fig. 5b), with no significant relation between imidacloprid concentrations and stream flow ($\rho = -0.05$, $P = 0.87$).

Streams in a nationally important ecosystem: Chesapeake Bay

For this research component, stream samples were collected from three agriculturally affected streams (Fig. 2c): Antietam Creek, Big Pipe Creek and Chillisquaque Creek, with a specific emphasis on collection of runoff samples during the planting season of cultivated crops. Overall, neonicotinoids were detected in 59 % of the stream samples. Clothianidin was the most frequently detected neonicotinoid (59 %), followed by thiamethoxam (29 %), and imidacloprid (6 %) (Table S4, Fig. 6). The thiamethoxam and imidacloprid detections were all found in the presence of clothianidin.

Similar to previous research on agricultural streams in the Midwestern USA,[11] an increase in neonicotinoid concentrations was observed in these streams during runoff conditions associated with the planting season of cultivated crops (Fig. 6). Although there were not enough samples to test statistically, the concentrations of clothianidin and thiamethoxam (the two neonicotinoids primarily used for agricultural purposes) generally increased as the amount of land used in cultivated crops increased. The highest concentrations for each site occurred on 16 May 2014 and sites ranked the same with respect to percentage cultivated crops and clothianidin plus thiamethoxam concentration: Big Pipe Creek (41 %; 93 ng L^{-1}) > Chillisquaque Creek (32 %; 64 ng L^{-1}) > Antietam Creek (21 %; 11 ng L^{-1}). The measurement of neonicotinoids is one component in determining if chemical exposures are contributing to fish health issues being observed in the Chesapeake Bay watershed. Future work will seek to determine the relative effects from various chemicals (e.g. neonicotinoids) and additive or synergistic

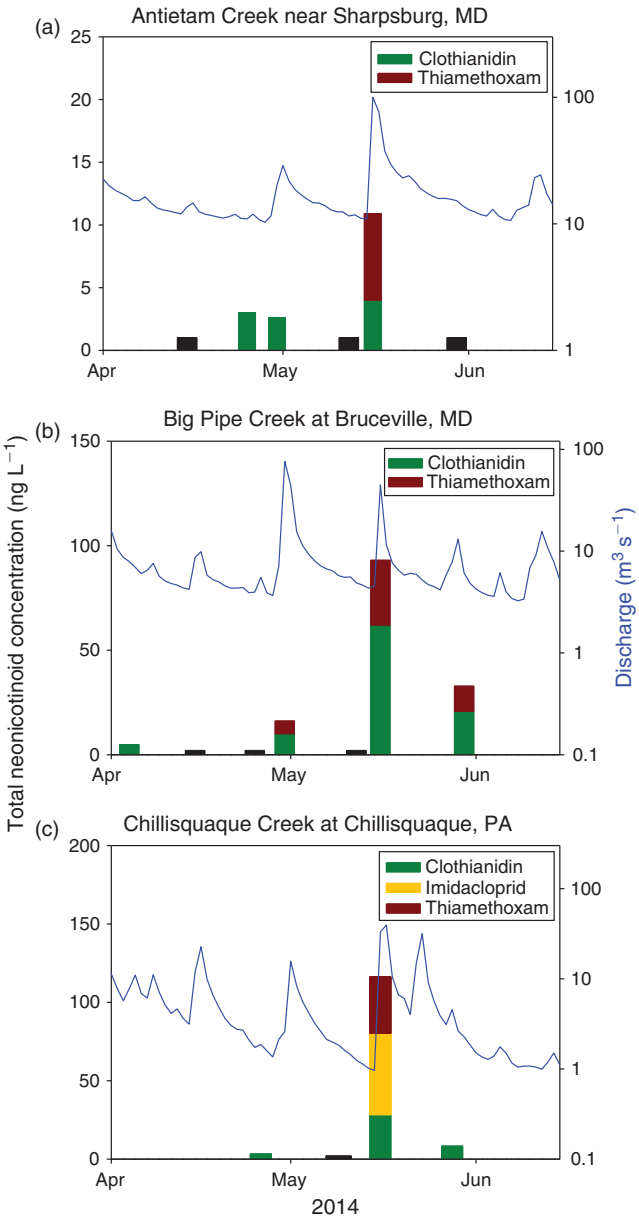

Fig. 6. Concentrations of clothianidin, imidacloprid and thiamethoxam and the corresponding stream discharge at three sites in the Chesapeake Bay area sampled in 2014. Black bars represent samples where no neonicotinoids were detected.

effects from chemical mixtures (e.g. pesticides, hormones, pharmaceuticals) on fish health.

In-stream transport: Fourmile Creek, Iowa

The two different sampling events of the 4.8-km study reach for this research component captured two vastly different WWTP-derived flow scenarios (99.5 and 11.0 % flow-derived effluent below the WWTP). The December 2012 sampling occurred during a time of prolonged drought conditions and months after any agricultural use of neonicotinoids had taken place. Thus, the flow of Fourmile Creek below the WWTP outfall was 99 % effluent at this time. Only imidacloprid and clothianidin were detected during this December sampling (Table S5). During this time, the input of WWTP effluent into Fourmile Creek caused an increase in stream concentration for imidacloprid and a decrease

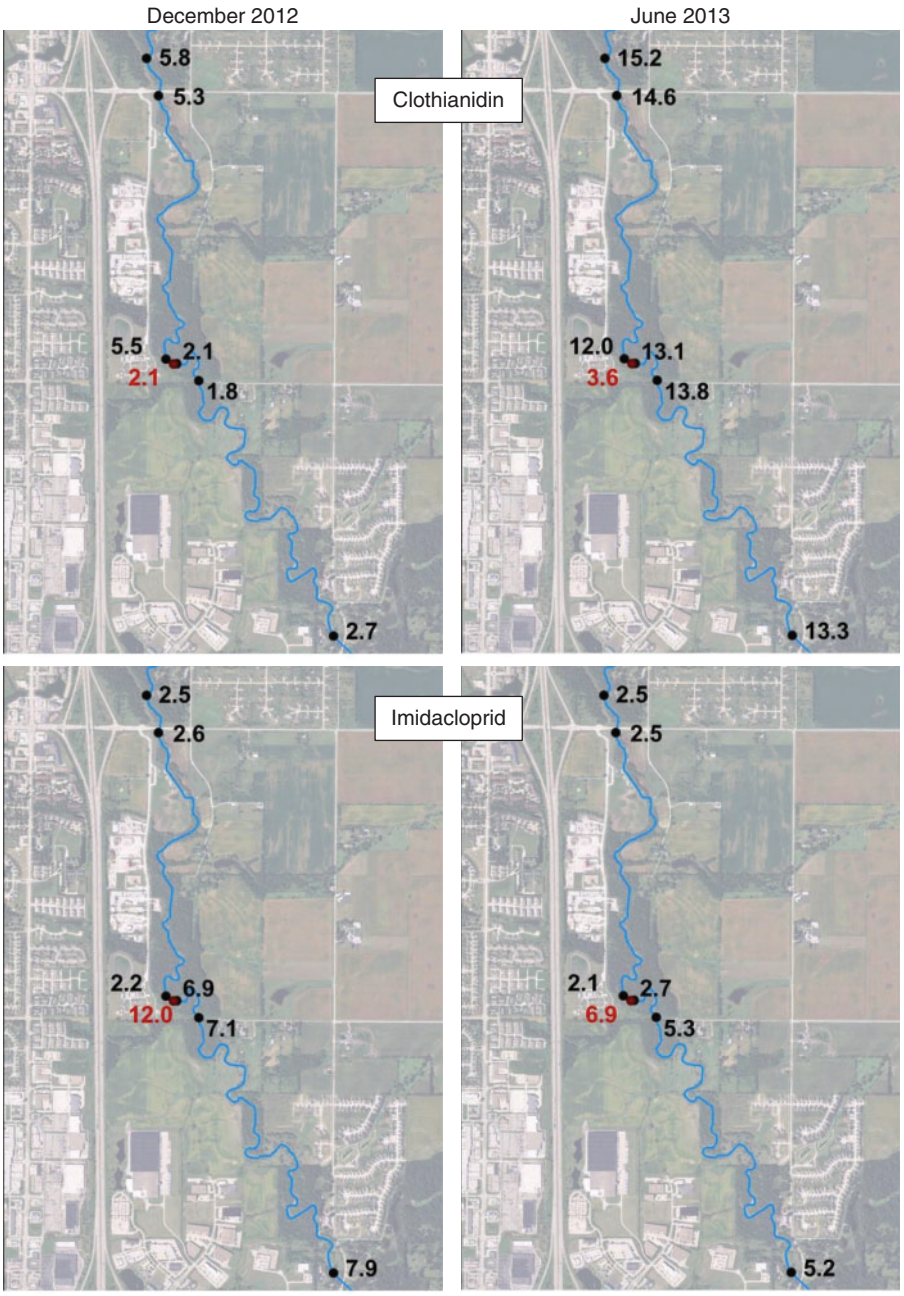

Fig. 7. Concentrations (ng L^{-1}) of clothianidin and imidacloprid collected along Fourmile Creek near Ankeny, Iowa, at two sampling times, December 2012 and June 2013. The concentrations in red are those found at the outfall of the wastewater treatment plant (WWTP).

in stream concentration for clothianidin (Fig. 7). This trend was not unexpected owing to the primary uses for these neonicotinoids (i.e. agricultural use for clothianidin and urban use for imidacloprid). These neonicotinoids appear to be transported conservatively through this study reach because similar concentrations were found from the uppermost sampling point to just above the WWTP outfall and from just below the WWTP outfall to the lowermost sampling point (Fig. 7).

The June 2013 sampling occurred during a time of normal, early-summer flow conditions (i.e. median flow) and soon after the primary period of the agricultural use of neonicotinoids had taken place in the headwaters. Correspondingly, the flow of Fourmile Creek below the WWTP outfall was 11 % effluent at this time. During this sampling, clothianidin, imidacloprid and thiamethoxam were detected (Table S5). Given the strong agricultural use for clothianidin and thiamethoxam, the higher stream concentrations in the upper portion of the reach, derived from row-crop production in the headwaters, compared with that observed during the December 2012 sampling were expected. As was observed previously, only clothianidin and imidacloprid were detected in the WWTP effluent, with clothianidin being present at lower concentrations than imidacloprid. In addition, the input of WWTP effluent into Fourmile Creek again caused an increase in stream concentrations for imidacloprid and a slight decrease in stream concentrations for clothianidin (Fig. 7). This trend, however, was dampened by the much lower influence of the WWTP on stream flow below the outfall (11 % effluent-derived stream flow) compared with the December

2012 sampling (99 % effluent-derived stream flow). As with the December sampling, conservative transport of neonicotinoids was observed, with similar concentrations from the uppermost sampling point to just above the WWTP outfall and from just below the WWTP outfall to the lowermost sampling point (Fig. 7).

Summary

In this first wide-scale investigation of neonicotinoids, they were frequently detected in streams across the USA, with 63 % of the 48 streams samples having a detection of at least one neonicotinoid. Both urban and agricultural uses contributed to stream neonicotinoid concentrations, with imidacloprid occurrence significantly related to the amount of urban land-use and clothianidin and thiamethoxam significantly related to the amount of cultivated crops. Similarly to previous research, transport to streams in agriculturally affected basins is driven by use and precipitation. The present study, however, has documented this to be true even when precipitation is heavy enough to cause substantial flooding. Research within a 4.8-km study reach of Fourmile Creek found that the input of WWTP effluent into the system caused stream concentrations of imidacloprid to increase and clothianidin to decrease. Both neonicotinoids, however, were found to be transported conservatively throughout the study reach. Although the present research provides important baseline data on neonicotinoid concentrations in streams and helps expand our understanding of their sources and environmental fate, more research is needed to understand the potential direct effects to aquatic organisms and indirect effects to both aquatic and terrestrial organisms from these stream neonicotinoid concentrations.

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