Pilot Study to Evaluate Neonicotinoid Pesticides in New York and Pennsylvania Streams

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Anne Secord[1]

Kathleen Patnode[2]

U.S. Fish and Wildlife Service

[1] 3817 Luker Road, Cortland, New York 13045

[2] 110 Radnor Road, Suite 101, State College, PA 16801

Abstract

The New York and Pennsylvania Field Offices of the U.S. Fish and Wildlife Service conducted a pilot study in 2015 and 2016 to evaluate whether neonicotinoid pesticides were present in streams in these states. Neonicotinoids are commonly used insecticides that persist in soil and may readily leach into streams, posing a threat to non-target invertebrates such as aquatic invertebrates and pollinators such as butterflies and bumblebees. Neonicotinoid pesticides were detected in the surface water of five of eleven streams sampled in New York and Pennsylvania, generally exceeding at least one toxicity benchmark for aquatic invertebrates (chronic USEPA water quality standard, EC10 or macrofauna abundance threshold). Thiacloprid was detected in one of five crayfish samples (New York) and imidacloprid was detected in one of ten freshwater mussel samples (Pennsylvania). Data from this limited pilot study indicate that neonicotinoids are present in surface water of streams in New York and Pennsylvania at concentrations that are consistent with concentrations detected in streams in other parts of the United States and Canada. Neonicotinoid detections in crayfish and freshwater mussels are evidence of bioaccumulation of these compounds. Further study is warranted to evaluate the significance of these compounds to aquatic ecosystem impairment and impacts to non-target terrestrial pollinator species.

Preface

In 2015 and 2016, the New York and Pennsylvania Field Offices of the U.S. Fish and Wildlife Service (USFWS) conducted a pilot study to evaluate neonicotinoids in streams. Our goal was to determine whether these commonly used insecticides are found in New York or Pennsylvania streams adjacent to or downstream from likely agricultural sources such as corn, soybeans, orchards, and potatoes. We are interested in understanding the occurrence of these pesticides in the environment, given their known or possible toxicity to aquatic invertebrates, as well as pollinators (butterflies and bees), and birds.

Properties of Neonicotinoids

Neonicotinoids are neuro-active insecticides that act mainly via binding strongly to nicotinic acetylcholine receptors on the cell membrane, causing an impairment of normal nerve impulses. They exhibit selective toxicity for insects over vertebrates (Matsuda et al. 2005). Neonicotinoids are moderately to highly soluble in water and systemically transported in plant tissues, making many parts of the plant toxic to feeding invertebrates (Bonmatin et al. 2015). Neonicotinoids include the compounds imidacloprid, thiamethoxam, acetamiprid, clothianidin, dinotefuran, nithiazine, nitenpyram, and thiacloprid.

Neonicotinoids are used as foliar or soil sprays, trunk injections, and to treat seeds prior to planting (Jeschke et al. 2011). The broad use of these compounds as seed treatments has resulted in unforeseen environmental releases, in that approximately 1 - 20% of the neonicotinoid applied as a seed treatment ends up in the crop, with the remainder entering the soil or lost as dust during sowing (Goulson 2013). This results in neonicotinoids ending up in soil, the air column, and in all parts of the growing plant (both crops and wild plants growing in agricultural margins). They may have substantial half-lives in soil, ranging from 200 to > 1000 days (Goulson 2013).

The nearly irreversible binding of imidacloprid to nicotinic acetylcholine receptors in insects results in broad toxicity to agricultural insect pests (http://npic.orst.edu/factsheets/archive/imidacloprid.html#references).
However, neonicotinoids have also been shown to be toxic to nontarget invertebrates, such as butterflies (Pecenka and Lundgren 2015; Krischik et al. 2015; Forister et al. 2016), lady beetles (Krischik et al. 2015), bees (Goulson 2015; Rundlof et al. 2015; Stanley and Raine 2017; Whitehorn et al. 2012; Godfray et al. 2014), and aquatic invertebrates (Roessink et al. 2013; Van Dijk et al. 2013).

Neonicotinoids have also been shown to adversely affect birds. For example, Eng et al. (2017) dosed white-crowned sparrows (*Zonotrichia leucophrys*) with imidacloprid and evaluated migration ability and body condition. They concluded that wild songbirds consuming the equivalent of four imidacloprid-treated canola seeds per day over three days could suffer impaired body condition, migration delays, and improper migratory direction. Lopez-Antia et al. (2015) fed imidacloprid-treated seeds to red-legged partridges (*Alectoris rufa*). The high dose (representing the recommended application rate) killed all partridges and the low dose (20% of the recommended application rate) impacted reproduction and depressed T-cell immune response in chicks.

History of Neonicotinoid Use

Neonicotinoids were developed in the 1980s and have rapidly become one of the most widely used insecticides in the world (Goulson 2013). Douglas and Tooker (2015) estimated that between 79 and 100% of corn hectares in the United States were treated with a neonicotinoid seed dressing in 2011. Neonicotinoids are most commonly used on soybeans, corn, cotton, grapes, and orchards (https://water.usgs.gov/nawqa/pnsp/usage/maps). Figure 1 illustrates the dramatic increase in imidacloprid use in the United States between 1995 and 2014.

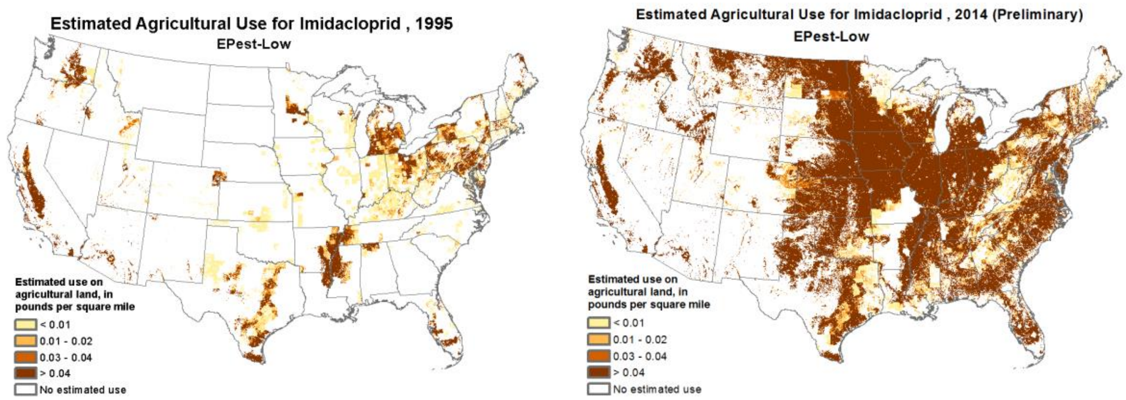

Figure 1. Comparison of Imidacloprid Use Between 1995 and 2014
(https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2014&map=IMIDACLOPRID&hilo=L&disp=Imidacloprid)

Neonicotinoids in the Environment

Neonicotinoids in Surface Water

Neonicotinoids are soluble in water, with solubilities ranging from 184 mg/L (moderate) to 590,000 mg/L (high) for imidacloprid and nitenpyram, respectively (PPDB 2012). Concentrations in surface water samples from the environment generally range from 0.001 parts per billion (ppb) to the low ppbs (Wood and Goulson 2017; Starner and Goh 2012; Hladik et al. 2014; Schaafsma et al. 2015; Benton 2016) (Table 1). The compounds have been detected in a variety of surface waters (e.g., streams, wetlands, ditches) across the United States and Canada, including in early spring prior to planting (Schaafsma et al. 2015; Main et al. 2014), a reflection of both the off-site transport and persistence of these compounds in surface waters (Table 1).

Table 1. Concentrations of Neonicotinoids in Water Samples from Various Locations			
Compound	**Concentration in ppb**	**Location**	**Citation**
Clothianidin	0.008 median/0.257 maximum (in 75% of samples overall)	Midwestern U.S. streams	Hladik et al. 2014
Clothianidin & thiamethoxam (total)	ND- 7.54 pre-planting/0.67 – 11.07 4-5 weeks post-planting	Canada adjacent to maize fields (puddles, ditches, drains)	Schaafsma et al. 2015
Imidacloprid	<0.002 median/0.0427 maximum (in 23% of samples overall)	Midwestern U.S. streams	Hladik et al. 2014
Imidacloprid	ND – 3.29 (in 89.3% of samples)	California rivers, creeks, and drains	Starner and Goh 2012
Imidacloprid	0.028 - 0.379	Appalachian mountain streams adjacent to treated forests	Benton 2016
Thiamethoxam	0.001 mean/0.017 maximum	Canada adjacent to maize fields (puddles, ditches, drains)	Schaafsma et al. 2015
Thiamethoxam	<0.002 median/0.185 maximum (in 47% of samples overall)	Midwestern U.S. streams	Hladik et al. 2014

Neonicotinoids in Soil/Sediment

Neonicotinoids may persist in soils from days to years, depending on the individual compound (Goulson 2013; Bonmatin et al. 2015). Studies have shown that an annual cycle of neonicotinoid use may result in chronic concentrations of these compounds in agricultural and adjacent soils in the range of 3.5 – 13.3 ppb for clothianidin and 0.4 – 4.0 ppb for thiamethoxam (Wood and Goulson 2017). Main et al. (2014) found neonicotinoid residues in 6% of wetland sediment samples from wetlands situated in agricultural fields from Canada's prairie pothole region, a demonstration of off-site transport from agricultural fields.

Neonicotinoids in Crop and Wild Plants

Agricultural use of neonicotinoids generally results in < 10 ppb in pollen and nectar of crop plants, with concentrations in pollen generally greater than concentrations in nectar (Wood & Goulson 2017). Average maximum neonicotinoid concentrations from 20 published studies were 1.9 ppb in nectar and 6.1 ppb in pollen of treated flowering crops (Godfray et al. 2014).

Neonicotinoids are also frequently found in wild plants growing in and around agricultural fields (Wood & Goulson 2017). For example, Botias et al. (2015) found 1.19 ppb and 0.51 ppb of imidacloprid and thiamethoxam, respectively, in wild plants near neonicotinoid seed-treated crops. Stewart et al. (2014) found average neonicotinoid concentrations of 9.6 ppb in wildflowers adjacent to maize fields in the southeastern United States. In summary, neonicotinoids may be found in all parts of crop plants, with concentrations of the various neonicotinoid pesticides in crops and wild plants generally in the low ppb range (Table 2). Insects that forage on or collect agricultural or wild plant materials, including nectar and pollen, may be exposed to neonicotinoids.

Table 2. Neonicotinoid Concentrations in Crop and Wild Plants

Compound	Location	Matrix	Concentration (ppb)	Citation
Clothianidin	Brookings Co., South Dakota	Milkweed next to corn fields	Mean = 1.14; Max = 4	Pecenka & Lundgren 2015
Clothianidin	East Sussex, England	Oilseed rape plants	Mean = 2.91	Botias et al. 2016
Imidacloprid	East Sussex, England	Oilseed rape plants	Mean = 0.23	Botias et al. 2016
Thiamethoxam	East Sussex, England	Oilseed rape plants	Mean = 1.04	Botias et al. 2016
Clothianidin	East Sussex, England	Wild plants near oilseed rape foliage	Mean = 0.51	Botias et al. 2016
Imidacloprid	East Sussex, England	Wild plants near oilseed rape foliage	Mean = 1.19	Botias et al. 2016
Thiamethoxam	East Sussex, England	Wild plants near oilseed rape foliage	Mean = 8.71	Botias et al. 2016
Clothianidin	Sweden	Field border plants – 2 weeks after sowing	Mean = 1.0	Rundlof et al. 2015
Clothianidin	NW Indiana	Maize pollen	Mean = 3.9	Krupke et al. 2012
Thiamethoxam	NW Indiana	Maize pollen	Mean = 1.7	Krupke et al. 2012

Methods

In June 2015, surface water and sediment samples were collected in streams downstream of crops such as corn, soybeans, grapes, and apple orchards in New York and Pennsylvania. Eight surface water and sediment samples from Pennsylvania and nine surface water and sediment samples from New York were collected and submitted for neonicotinoid analysis (acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam). Aquatic invertebrates were collected at sites, if present in sufficient quantities. An additional single surface water sample (7C) was also collected in Pennsylvania in September 2015.

One liter of water was collected from the top 15 cm of the water column. Sediment grab samples of approximately 500 grams were collected from the top 25 cm of the sediment column using cleaned stainless steel spoons. Aquatic invertebrates were collected using kick nets. Invertebrate samples were dominated by crayfish, with a very small contribution (by mass) of snails and worms. All samples were stored in chemically cleaned jars and kept refrigerated (water) or frozen (sediment and tissue samples) until submittal to the laboratory.

Sampling was conducted in July of 2016 in a separate set of streams in New York and Pennsylvania, with an emphasis on targeting streams downstream or adjacent to potato fields (Figure 2). Seventeen sediment samples (from 16 separate sites) and 11 surface water samples were analyzed for neonicotinoids. We also submitted 10 samples of freshwater mussel tissue collected in 2016 from Pennsylvania streams and five samples of mixed aquatic invertebrates (largely crayfish) collected from New York in 2015.

Results

No neonicotinoids were detected in water or sediment samples collected in June 2015. We later determined that the laboratory Reporting Limit of 3 ppb for both water and sediment was unreasonably high (e.g., ~ 10X the U.S. Environmental Protection Agency (USEPA) acute freshwater aquatic toxicity benchmark). **We determined the June 2015 sediment and surface water data to be invalid due to the high detection limit.** The laboratory was subsequently able to reduce the Reporting Limit to 0.002 ppb for surface water. A single surface water sample (7C) collected in Pennsylvania in September 2015 (downstream of potato fields) was submitted for analysis in late 2015. Imidacloprid was detected at 0.002 ppb in that sample (Table 3 and Figure 2).

Whereas in 2015, we sampled in streams downstream of a variety of crops that are characteristically treated with neonicotinoids, our goal with the 2016 sampling was to focus on streams downstream of potato fields because imidacloprid had been detected in a single 2015 surface water sample that was associated with potatoes. We were also working with the laboratory to improve their Reporting Limits. The laboratory was able to achieve a 0.004 ppb Reporting Limit for the 2016 surface water samples. Five 2016 surface water samples (from 4 separate sites in New York State) had detectable concentrations of neonicotinoids ranging from 0.005 ppb to 0.319 ppb (Table 3 and Figure 2). Neonicotinoids were not detected in six other surface water samples from New York and Pennsylvania.

The laboratory was only able to achieve a Reporting Limit of 3 ppb for 2016 sediment and biological tissue. No neonicotinoids were detected in 2016 sediment samples using this limited analytical protocol.

Imidacloprid was detected in one of ten 2016 mussel samples from French Creek, Pennsylvania (6.31 ppb) and thiacloprid was detected in a June 2015 New York multiple-stream composite of mixed aquatic invertebrates dominated by crayfish (7.33 ppb). No neonicotinoids were detected in nine samples of mussel tissue from French Creek in Pennsylvania and four samples of mixed invertebrates from New York; however, we note again the relatively insensitive Reporting Limit of 3 ppb for tissue samples.

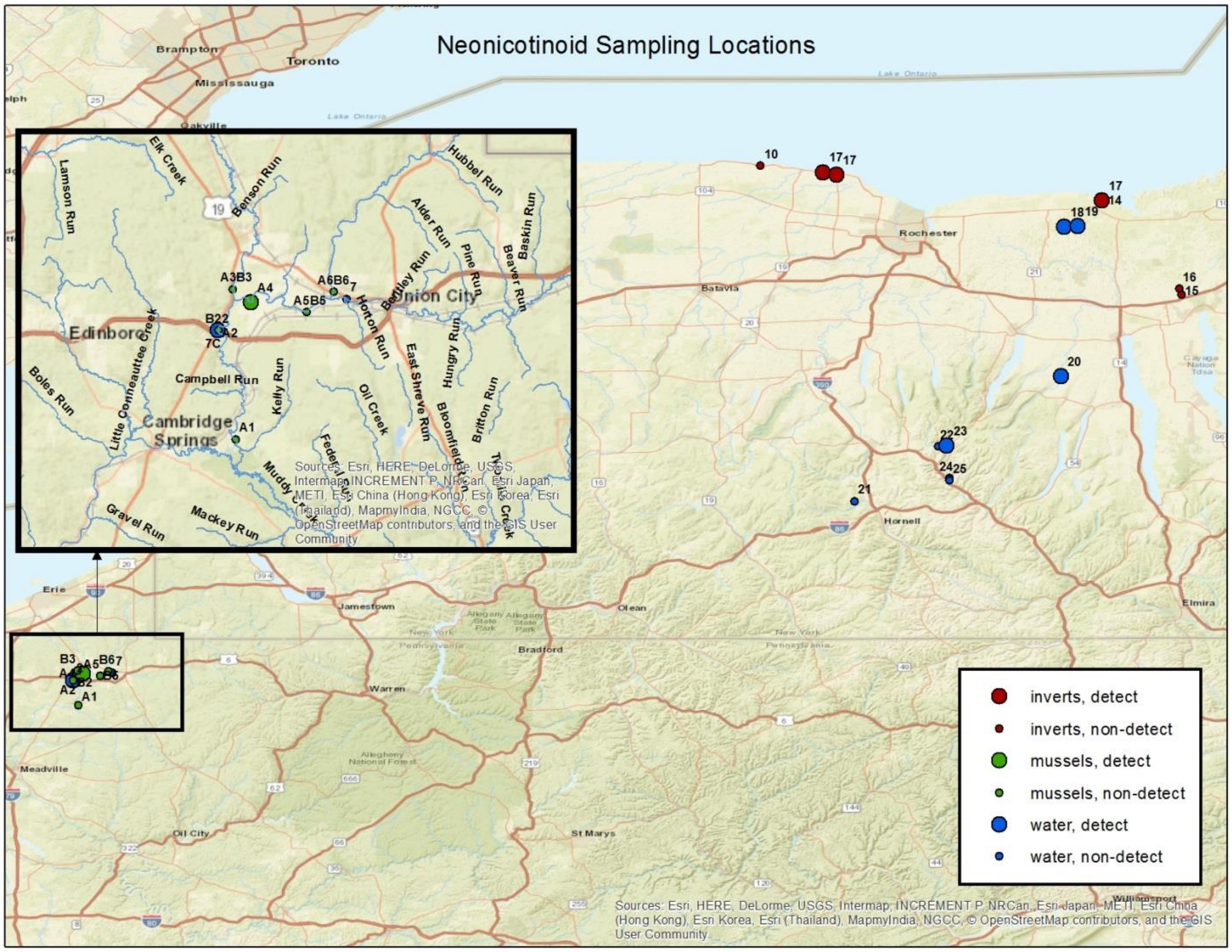

Figure 2. Neonicotinoid Sampling Locations

FLINT CREEK, YATES COUNTY, NY

SALMON CREEK, WAYNE COUNTY, NY

Figure 3. Photos of Selected Sample Locations

Table 3. Results of Neonicotinoid Sampling in NY and PA Streams

Site	Matrix	Compound	Concentration (ppb ww)	Notes on Location
2015 Samples				
SW7C	SW*(surface water)	Imidacloprid	0.002 (reporting limit 0.002 ppb)	French Creek, PA - Downstream of potatoes
NewNeoBug**10**	Mostly crayfish	All analytes	<3 ppb (reporting limit)	Bald Eagle Creek, Orleans County, NY – Downstream of corn, beans, orchards
NewNeoBug**14**	Mostly crayfish	All analytes	< 3 ppb	Maxwell Creek, Wayne County, NY – Downstream of orchards
NewNeoBug**15**	Mostly crayfish	All analytes	< 3 ppb	Black Brook, Cayuga County, NY –Downstream of corn, beans
NeoNeoBug**16**	Mostly crayfish	All analytes	< 3 ppb	White Brook, Cayuga County, NY Downstream of corn, beans
NewNeoBug**17**	Mostly crayfish	Thiacloprid	7.33 (18.94 dw) (reporting limit 3 ppb)	Composite sample from East Creek and Cowsucker Creek (Monroe Co) & Sill Creek (Wayne Co) NY - Downstream of orchards, corn
2016 Samples				
FCSW02	SW*(surface water)	All analytes	<0.004 (reporting limit)	French Creek, Erie County, PA – Downstream of soybeans
FCSW07	SW	All analytes	<0.004	French Creek, Erie County, PA -Downstream of corn
NYP18	SW	Acetamiprid	0.005	Salmon Creek 1, Wayne County, NY - Downstream of potatoes, corn, beans, orchards
NYP18	SW	Imidacloprid	0.114	
NYP19	SW	Imidacloprid	0.319	Salmon Creek 2, Wayne County, NY - Downstream of corn, beans, potatoes, orchards
NYP19Dupe	SW	Imidacloprid	0.300	Salmon Creek 2, Wayne County, NY - Downstream of corn, beans, potatoes, orchards
NYP19Dupe	SW	Thiamethoxam	0.005	

NYP20	SW	Imidacloprid	0.006	Flint Creek, Yates County - Downstream of potatoes, corn, beans
NYP21	SW	All analytes	<0.004	Marsh Ditch, Steuben County, NY –Downstream of potatoes, corn
NYP22	SW	All analytes	<0.004	Cohocton River, Steuben County – downstream potatoes, corn, beans
NYP23	SW	Imidacloprid	0.018	Cohocton River, Steuben County, NY - Downstream of potatoes, corn, beans, wetland
NYP24	SW	All analytes	<0.004	Cohocton River, Steuben County – Downstream of potatoes, corn, beans
NYP25	SW	All analytes	<0.004	Cohocton River, Steuben County – Downstream of potatoes, corn, beans
*All 2015 surface water samples (except SW7C – reporting limit of 0.002 ppb) had a Reporting Limit of 3 ppb and were considered invalid; all 2016 surface water samples had a Reporting Limit of 0.004 ppb				
FCMUSA01	Mussel	All analytes	< 3 ppb (reporting limit)	French Creek, PA – Downstream of soybeans
FCMUSA02	Mussel	All analytes	< 3 ppb	French Creek, PA – Downstream of soybeans
FCMUSA03	Mussel	All analytes	< 3 ppb	French Creek, PA – Downstream of soybeans
FCMUSA04	Mussel	Imidacloprid	6.31 (48.91 dw)	French Creek, PA – Downstream of soybeans
FCMUSA05	Mussel	All analytes	< 3 ppb	French Creek, PA – Downstream of soybeans, potatoes
FCMUSA06	Mussel	All analytes	< 3 ppb	French Creek, PA – Downstream of potatoes, corn
FCMUSB02	Mussel	All analytes	< 3 ppb	French Creek, PA – Downstream of soybeans
FCMUSB03	Mussel	All analytes	< 3 ppb	French Creek, PA – Downstream of soybeans, potatoes
FCMUSB05	Mussel	All analytes	< 3 ppb	French Creek, PA – Downstream of soybeans
FCMUSB06	Mussel	All analytes	< 3 ppb	French Creek, PA – Downstream of potatoes, corn

Discussion

Effects of Neonicotinoids in Water on Aquatic Invertebrates

Laboratory studies have determined that neonicotinoids at field relevant water concentrations may adversely impact aquatic invertebrates, as summarized in Table 4. For example, 0.03 ppb imidacloprid was determined to be the 28 day 10% Effects Concentration - EC10 (immobilization) for the mayfly *Caenis horaria* (Roessink et al. 2013). This concentration has been reported in surface waters in the Midwestern U.S., Appalachian streams, California surface waters, and in Salmon Creek (Wayne County) in New York (see Tables 1 & 3).

Van Dijk et al. (2013) evaluated imidacloprid concentrations in surface water as related to the abundance of aquatic macroinvertebrates in streams in the Netherlands. They found a significant negative relationship between macroinvertebrate abundance and imidacloprid concentration for all species pooled. Their data showed that macrofauna abundance dropped sharply between 0.013 and 0.067 ppb imidacloprid in surface water, a concentration range that was exceeded in two New York streams (Salmon Creek & Cohocton River).

The USEPA acute and chronic freshwater aquatic life benchmarks for imidacloprid to protect invertebrates are 0.385 ppb and 0.01 ppb, respectively. (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk). Multiple studies demonstrate that the USEPA chronic freshwater aquatic life benchmark for imidacloprid is frequently exceeded in U.S. surface waters, with some exceedences of the USEPA acute aquatic life benchmark (Table 1). The chronic USEPA standard for imidacloprid was exceeded in Salmon Creek and the Cohocton River in New York.

Table 4. Water Quality Endpoints for Neonicotinoids

Neonicotinoid	Water Conc (ppb)	Organism	Endpoint	Authors
Studies				
Imidacloprid	0.03	Mayfly	28 day EC10 immobilization	Roessink et al. 2013
Imidacloprid	0.1 – 0.3	Mayfly	96 hour EC10 immobilization	Roessink et al. 2013
Imidacloprid	0.2 – 0.3	Mayfly	28 day LC50 (lethal concentration 50%)	Roessink et al. 2013
Imidacloprid	0.532	Caddisfly	96 hour EC10 immobilization	Roessink et al. 2013
Thiacloprid	5.76	*Simulium latigonium*	24 hour LC50 for most sensitive organism	Beketov & Leiss 2008
Imidacloprid	0.013 – 0.067		Major drop in macroinvertebrate abundance	Van Dijk et al. 2013 (field data – Netherlands)

Standards & Benchmarks				
Imidacloprid	0.0083		Dutch chronic standard	Smit et al. 2015
Imidacloprid	0.20		Dutch acute standard	Smit et al. 2015
Imidacloprid	0.23 (active ingredient)		Canada CCME interim fresh water guideline	Canadian Council of Ministers of the Environment 2007
Imidacloprid	0.01		USEPA chronic freshwater aquatic life benchmark for invertebrates *	USEPA
Imidacloprid	0.385		USEPA acute freshwater aquatic life benchmark for invertebrates *	USEPA
Clothianidin	1.1		USEPA chronic freshwater aquatic life benchmark for invertebrates *	USEPA
Clothianidin	11		USEPA acute freshwater aquatic life benchmark for invertebrates *	USEPA
* https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk				

The challenges we experienced with insensitive laboratory reporting limits makes it difficult to draw conclusions from this data set, particularly the 2015 surface water samples and all sediment and tissue samples. With an appropriate reporting limit of 0.004 ppb, neonicotinoids were detected in the surface water of a number of streams (Salmon Creek, {Wayne County} NY, Flint Creek, NY, Cohocton River, NY; French Creek, PA). At three sample sites in New York, imidacloprid was detected in excess of the USEPA chronic freshwater aquatic life benchmark for invertebrates (0.01 ppb). Two water samples from Salmon Creek in Wayne County, New York, had imidacloprid concentrations that exceeded the EC10 for mayflies (0.03 ppb) and these samples, as well as a sample from the Cohocton River, exceeded a threshold associated with a drop in macroinvertebrate abundance (0.013 – 0.067 ppb) (Van Dijk et al. 2013). One sample from the Salmon River in Wayne County (0.319 ppb) approached the USEPA acute freshwater aquatic life benchmark for invertebrates (0.385 ppb).

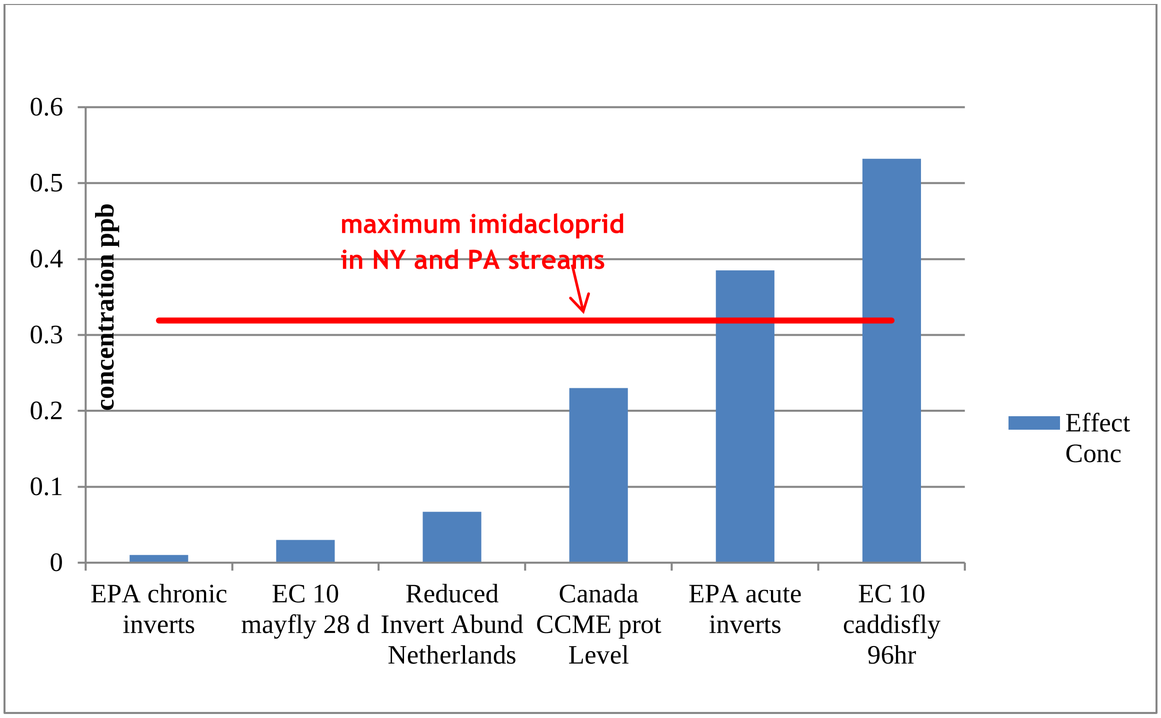

Figure 4. Imidacloprid in New York and Pennsylvania Streams (red line shows maximum concentration) Compared to Water Quality Endpoints for Imidacloprid.

Relevance of Neonicotinoids in Tissue Samples

One of five mixed invertebrate samples and one of ten freshwater mussel samples had detections of thiacloprid and imidacloprid, respectively, despite the high reporting limit.

We found that published neonicotinoid tissue data are limited for aquatic species. The U.S. Geological Survey (USGS) has analyzed benthic macroinvertebrate tissues from wetlands in North Dakota. The researchers found imidacloprid concentrations ranging from 13.79 ppb dw in Coenagrionidae (damselfly) larvae to 23.53 ppb dw in Dipteran larvae, as well as clothianidin from 3.96 to 18.14 ppb dw in Dipteran larvae (J. Kraus, pers. comm.). Our data (18.94 ppb dw thiacloprid in crayfish) demonstrate that crayfish accumulated neonicotinoids in the range of benthic macroinvertebrates sampled by USGS. We were unable to locate any toxicity or tissue data for neonicotinoids in any crayfish species.

The single finding of imidacloprid in a mussel sample in our study suggests that under certain conditions, these filter feeders can accumulate neonicotinoids. Lack of detectable concentrations in the collocated water sample suggests that mussels may be repeatedly exposed to low concentrations of neonicotinoids (that are not detectable with our laboratory reporting limits), possibly with metabolism and depuration occurring very slowly, allowing neonicotinoids to accumulate in mussel tissue. There is little information on the effects of neonicotinoids on mussels. Juvenile wavy-rayed lampmussels (*Lampsilis fasciola*) were found to survive high concentrations of neonicotinoids (7 day LC50 >456 ppb of imidacloprid, clothianidin, or thiamethoxam) in water (Prosser et al. 2016). This concentration greatly exceeds any of the surface water concentrations detected as part of this study.

Neonicotinoids were shown to bind to a molluscan nicotinic receptor in the pond snail (*Lymnaea stagnalis*), but suppressed (up to 95%) rather than increased nerve transmission as seen in insects (Vehovszky et al. 2015). Other pesticides that block nerve transmission (i.e., organophosphates and carbamates) have been shown to impair adductor muscle responsiveness in *Elliptio complanata* (Moulton et al. 1996). While not lethal in a laboratory testing, the inability to use adductor or foot muscles could impair long-term survival and reproduction in wild mussels that accumulate high concentrations of neonicotinoids. Further testing of mussel responses to neonicotinoids is warranted to determine if federally listed mussels are at risk from exposure to these pesticides.

Conclusions

Data from this pilot study, though limited by analytical deficiencies, demonstrate that neonicotinoids are found in streams in New York and Pennsylvania in excess of toxicity and regulatory thresholds and they may bioaccumulate in aquatic organisms. Further study with realistic analytical reporting limits is needed to evaluate the scope and significance of neonicotinoids in streams in New York and Pennsylvania. Potential avenues to explore would be to:

- Evaluate surface water concentrations and seasonality associated with various types of agriculture.
- Evaluate chronic and sublethal effects of neonicotinoids on mussels and crayfish to determine risk to federally listed species or other conservation priority species.
- Evaluate correlation between neonicotinoid concentrations and aquatic invertebrate abundance and diversity.
- Evaluate bioavailability of neonicotinoids by analyzing tissues of aquatic organisms in streams with high neonicotinoid use.
- Evaluate significance of neonicotinoid use to pollinators (see Appendix B).

Appendix A. Sample Locations and Data Reports

Neonicotinoid Sample Sites - 2015 & 2016						
Sample #	**Matrix**	**Coordinates**		**Location**	**Year Collected**	**Crops**
SW7C	water	41°52'56"N	79°59'59"W	French Creek, PA	2015	potatoes
2	water	41° 52' 56.1036'' N	79° 59' 57.768'' W	French Creek, PA	2016	soybeans
7	water	41° 54' 10.7352'' N	79° 53' 9.3552'' W	French Creek, PA	2016	corn
18	water	43,11,12.61	77,08,25.39	Salmon Creek, NY	2016	corn,beans,potatoes,orchards
19	water	43,11,19.60	77,06,6.15	Salmon Creek, NY	2016	corn,beans,potatoes,orchards
20	water	42,45,24.17	77,09,02.48	Flint Creek, NY	2016	potatoes,corn,beans
21	water	42,23,49.92	77,44,47.29	Marsh Ditch, NY	2016	potatoes,corn
22	water	42,33,17.09	77,30,19.01	Cohocton R, NY	2016	potatoes,corn,beans
23	water	42,33,24.52	77,28,46.2	Cohocton R, NY	2016	potatoes,corn,beans
24	water	42,27,50.61	77,28,20.17	Cohocton R, NY	2016	potatoes,corn,beans
25	water	42,27,25.02	77,28,18.20	Cohocton R, NY	2016	potatoes,corn,beans
A1	mussels	41° 48' 37.9224'' N	79° 59' 1.9464'' W	French Creek, PA	2016	soybeans
A2	mussels	41° 52' 56.1036'' N	79° 59' 57.768'' W	French Creek, PA	2016	soybeans
A3	mussels	41° 54' 34.0236'' N	79° 59' 10.896'' W	French Creek, PA	2016	soybeans
A4	mussels	41° 54' 2.8224'' N	79° 58' 14.3148'' W	French Creek, PA	2016	soybeans
A5	mussels	41° 53' 39.2964'' N	79° 55' 16.9932'' W	French Creek, PA	2016	potato/soybean
A6	mussels	41° 54' 28.2024'' N	79° 53' 50.1936'' W	French Creek, PA	2016	potato/corn
B2	mussels	41° 52' 56.1036'' N	79° 59' 57.768'' W	French Creek, PA	2016	soybeans
B3	mussels	41° 54' 34.0236'' N	79° 59' 10.896'' W	French Creek, PA	2016	soybeans
B5	mussels	41° 53' 39.2964'' N	79° 55' 16.9932'' W	French Creek, PA	2016	potato/soybean
B6	mussels	41° 54' 28.2024'' N	79° 53' 50.1936'' W	French Creek, PA	2016	potato/corn
10	inverts	43° 21' 50.38'' N	78°01'00.21"W	Bald Eagle Creek, NY	2015	corn, beans, orchards
14	inverts	43°15'53.19"N	77°01'28.10"W	Maxwell Creek, NY	2015	orchards
15	inverts	42°,59',30.77"N	76°,48",11.68"W	Black Brook, NY	2015	corn,beans
16	inverts	43°00'33.68"N	76°48'34.69"W	White Brook, NY	2015	corn,beans
17	inverts	43°20'40.89" N	77°50'13.25"W	Cowsucker Creek, NY	2015	orchards,corn
17	inverts	43°20'12.93" N	77°47'54.48"W	East Creek, NY	2015	orchards,corn
17	inverts	43°15'45.04"N	77°01'54.85"W	Sill Creek, NY	2015	orchards,corn
Shaded samples were positive for neonicotinoids; sample 17 was a composite sample from three creeks; invertebrate samples were dominated by mass by crayfish						

WATER 2015				MISSISSIPPI STATE CHEMICAL LABORATORY
SAMPLE TYPE:	Water/Sediment			BOX CR
Project: FWS Neonicotinoids				MISS. STATE, MS 39762
				REPORT FORM
CAT # : 5050084				
				Neonicotinoids
				PARTS PER BILLION AS RECEIVED (WET WT)
		MDL**		
FWS #			**SW7C**	
LAB #			151001001-001	
MATRIX			Water	
COMPOUND				
Acetamiprid		0.002	*ND	
Clothianidin		0.002	ND	
Imidacloprid		0.002	0.002	
Thiacloprid		0.002	ND	
Thiamethoxam		0.002	ND	
		Reporting		
FWS #		Limit	SED7C	
LAB #			151001001-002	
MATRIX			Sediment	
COMPOUND				
Acetamiprid		3.0	*ND	
Clothianidin		3.0	ND	
Imidacloprid		3.0	ND	
Thiacloprid		3.0	ND	
Thiamethoxam		3.0	ND	
*ND = None Detected				
** MDL = Method Detection Limit				

WATER 2016				MISSISSIPPI STATE CHEMICAL LABORATORY				Page 1
SAMPLE TYPE:	Water/Tissue/Sediment			BOX CR				
Project: FWS Neonicoinoid				MISS. STATE, MS 39762				
				REPORT FORM				
CAT # : 5050084							Spl Recd	7/26/2016
				Neonicotinoids				
				PARTS PER BILLION AS				

				RECEIVED (WET WT)				
		Reporting						
FWS #		Limit	FCSW02	FCSW07	NYP18SW	NYP19SW	NYP19SWDupe	NYP20SW
LAB #			160801001-001	160801001-002	160801001-003	160801001-004	160801001-005	160801001-006
MATRIX			Water	Water	Water	Water	Water	Water
COMPOUND								
Acetamiprid		0.004	*ND	ND	0.005	ND	ND	ND
Clothianidin		0.004	ND	ND	ND	ND	ND	ND
Imidacloprid		0.004	ND	ND	0.114	0.319	0.300	0.006
Thiacloprid		0.004	ND	ND	ND	ND	ND	ND
Thiamethoxam		0.004	ND	ND	ND	ND	0.005	ND
		Reporting						
FWS #		Limit	NYP21SW	NYP22SW	NYP23SW	NYP24SW	NYP25SW	
LAB #			160801001-007	160801001-008	160801001-009	160801001-010	160801001-011	
MATRIX			Water	Water	Water	Water	Water	
COMPOUND								
Acetamiprid		0.004	ND	ND	ND	ND	ND	
Clothianidin		0.004	ND	ND	ND	ND	ND	
Imidacloprid		0.004	ND	ND	0.018	ND	ND	
Thiacloprid		0.004	ND	ND	ND	ND	ND	
Thiamethoxam		0.004	ND	ND	ND	ND	ND	
*ND = None Detected								

INVERTEBRATES				MISSISSIPPI STATE CHEMICAL LABORATORY				Page 2
SAMPLE TYPE:	Water/Tissue/Sediment			BOX CR				
Project: FWS Neonicoinoid				MISS. STATE, MS 39762				
				REPORT FORM				
CAT # : 5050084							Spl Recd	7/26/2016
				Neonicotinoids				
				PARTS PER BILLION AS RECEIVED (WET WT)				
		Reporting						
FWS #		Limit	FCMUSA01	FCMUSA02	FCMUSA03	FCMUSA04	FCMUSA05	FCMUSA06
LAB #			160801001-012	160801001-013	160801001-014	160801001-015	160801001-016	160801001-017
MATRIX			Tissue	Tissue	Tissue	Tissue	Tissue	Tissue

COMPOUND								
Acetamiprid		3.0	*ND	ND	ND	ND	ND	ND
Clothianidin		3.0	ND	ND	ND	ND	ND	ND
Imidacloprid		3.0	ND	ND	ND	6.31	ND	ND
Thiacloprid		3.0	ND	ND	ND	ND	ND	ND
Thiamethoxam		3.0	ND	ND	ND	ND	ND	ND
		Reporting						
FWS #		Limit	FCMUSB02	FCMUSB03	FCMUSB05	NewNeoBug 10	FCMUSB0 6	NewNeoBug 14
LAB #			160801001-018	160801001-019	160801001-020	160801001-021	16080100 1-022	160801001-023
MATRIX			Tissue	Tissue	Tissue	Tissue	Tissue	Tissue
COMPOUND								
Acetamiprid		3.0	ND	ND	ND	ND	ND	ND
Clothianidin		3.0	ND	ND	ND	ND	ND	ND
Imidacloprid		3.0	ND	ND	ND	ND	ND	ND
Thiacloprid		3.0	ND	ND	ND	ND	ND	ND
Thiamethoxam		3.0	ND	ND	ND	ND	ND	ND
		Reporting						
FWS #		Limit	NewNeoBug 15	NewNeoBug 16	NewNeoBug 17			
LAB #			160801001-024	160801001-025	160801001-026			
MATRIX			Tissue	Tissue	Tissue			
COMPOUND								
Acetamiprid		3.0	ND	ND	ND			
Clothianidin		3.0	ND	ND	ND			
Imidacloprid		3.0	ND	ND	ND			
Thiacloprid		3.0	ND	ND	7.33			
Thiamethoxam		3.0	ND	ND	ND			
*ND = None Detected								

Appendix B. Effects of Neonicotinoids on Pollinators

Butterflies

Population Studies

Butterflies have been hypothesized to exhibit reduced numbers and population effects in association with neonicotinoid use. For example, Forister et al. (2016) found an association between butterfly declines in lowland Northern California and increasing neonicotinoid use, while controlling for land use and other factors. Gilburn et al. (2015) tested models to try to understand the 58% decline in the abundance of butterflies on farmed land in England from 2000 and 2009. They determined that the number of hectares of farmland where neonicotinoid pesticides are used is negatively associated with butterfly abundance. Further research is needed to determine whether neonicotinoids are causing the butterfly decline or whether other factors associated with intensive agriculture may be responsible.

Laboratory Studies

Imidacloprid applied to soil at 300 mg/pot resulted in concentrations in milkweed of 6.03 ppm (6,030 ppb) (Table B1). Lady beetles fed the milkweed flowers had lower survival. Adult butterfly (monarch and painted lady) survival was not reduced when fed the imidacloprid treated milkweed, but survival of butterfly larvae was significantly reduced. Typical concentrations of imidacloprid and other neonicotinoids in crops and adjacent vegetation have generally been in the low ppb range (Pecenka and Lundgren 2015 – see Table 2), suggesting that the 6,030 ppb imidacloprid detected in milkweed from this experiment is a concentration that is much greater than might be detected in vegetation consumed by insects in the wild.

Pecenka and Lundgren (2015) fed clothianidin-treated leaves to first instar monarch butterfly caterpillars. They calculated 36 hour LC_{10}, LC_{20} and LC_{50} values of 7.72 ppb, 9.89 ppb, and 15.63 ppb. Sublethal effects on growth (reduced body length and development rate of first instar) were found at dietary concentrations of 0.5 ppb clothianidin. Milkweed plants sampled adjacent to corn fields contained a mean clothianidin concentration of 1.14 ppb, with a maximum concentration of 4 ppb. The authors concluded that monarch larvae may be exposed to clothianidin in the field at potentially harmful concentrations.

Most recently, Whitehorn et al. (2018) fed farmland butterflies (*Pieris brassicae*) cabbage plants that had been watered with 0, 1, 10, 100 and 200 ppb imidacloprid (assumed to represent field margin plants growing in contaminated agricultural soils). They found that the duration of pupation and size of adult butterflies were both significantly reduced in the exposed butterflies compared to controls at all concentrations used. There was no effect of treatment on butterfly behavior. Although this study provides evidence of imidacloprid adversely affecting butterfly fitness, it is unclear how the concentrations of imidacloprid used to water the cabbage translate into forage or soil concentrations of imidacloprid.

The Pecenka and Lundgren (2015) and Whitehorn et al. (2018) data demonstrate that butterflies feeding on neonicotinoid-treated crop or wild plants may ingest concentrations that could potentially impair growth and development. These laboratory studies provide empirical support for the observations of reduced butterfly abundance in California and England, although more study is needed to confirm a causal link between neonicotinoid use and reduced butterfly numbers.

Table B1. Effects of Neonicotinoids on Terrestrial Invertebrates				
Compound	Conc/matrix	Species/Life Stage	Response	Citation
Butterflies and Moths				
Imidacloprid applied to soil in greenhouse	6,030 ppb in milkweed flowers	Monarch & painted lady larvae affected but adult survival not affected	Significantly reduced survival of larvae	Krischik et al. 2015
Imidacloprid, as above	6,030 ppb in milkweed flowers	Lady beetle	Significantly reduced survival	Krischik et al. 2015
Clothianidin	7.72 ppb/milkweed flowers	Monarch larvae	LC20	Pecenka & Lundgren 2015
Clothianidin	15.63 ppb/milkweed leaves	Monarch larvae	LC50	Pecenka & Lundgren 2015
Clothianidin	0.5 ppb/milkweed leaves	Monarch larvae	Reduced body length of first instar	Pecenka & Lundgren 2015
Imidacloprid	0,1,10,100,200 ppb in water applied to soil growing cabbage plants	Farmland butterfly pupae and adults	Reduced duration of pupation; reduced adult body size	Whitehorn et al. 2018
Bees				
Clothianidin	5 ppb in sucrose solution fed for 5 weeks	Bumblebee	Fewer workers, drones, and gynes after 35 days	Arce et al. 2017
Thiamethoxam	5.32 ppb in syrup for 14 days	Bumblebee (4 species)	Reduced feeding & impacts to ovary development	Baron et al. 2017
Thiamethoxam	2.4 & 10 ppb in sugar water solution for up to 27 days	Bumblebee	No impact on colony weight gain, or number or mass of sexuals produced	Stanley & Raine 2017

Bees

Bees {honeybees (*Apis mellifera*) and bumblebees (*Bombus spp*.) of the Apidae family} bring pollen and nectar to their hives to feed larvae and may be exposed to neonicotinoids from these plant sources. The degree of exposure and threat to bees depends on many factors, including types of plants used for foraging, mixture of agricultural and non-agricultural plant sources, seasonal exposure, and level of insecticide contamination. An analysis by Godfray et al. (2014) hypothesized that, in many circumstances, the daily intake of neonicotinoids by nectar and pollen foraging honeybees would typically be no more than a few percentage of the acute oral LD_{50} (Godfray et al. 2014).

Bumblebees may be more sensitive to neonicotinoids than honeybees. Bumblebees fed solutions spiked with 5 ppb clothianidin exhibited only subtle changes in foraging behavior, but the treated colonies showed fewer individuals after 5 weeks (Arce et al. 2017). In another experiment with four species of bumblebees, queens were fed a solution of thiamethoxam for 14 days at concentrations of 1.87 ppb and 5.32 ppb. The higher concentration caused 2 out of 4 species to reduce feeding and reduced the average length of terminal oocytes in all 4 species (Baron et al. 2017). Stanley and Raine (2017) found that neither 2.4 ppb nor 10 ppb of thiamethoxam fed to bumblebees for 27 days had an impact on colony growth or sexual production.

In general, studies have shown that non-lethal doses of neonicotinoids may affect the performance of wild bees (e.g., bumblebees) and pollinator colonies, but there has been debate about whether the concentrations tested are realistic field concentrations (Wood and Goulson 2017).

Conclusion

Studies have identified the potential for pollinating insects such as bees and butterflies to be exposed to concentrations of neonicotinoids that may impact feeding, growth, and reproduction. It is important to evaluate the impacts of these compounds on pollinators at realistic field concentrations, particularly looking at the effects on species of special concern, such as the bog buckmoth (*Hemileuca maia*) (NY endangered), frosted elfin (*Callophrys irus*) (NY threatened), yellow-banded bumblebee (*Bombus terricola*) (USFWS Species of Concern), rusty patched bumblebee (*Bombus affinis*) (federal endangered), and monarch butterfly (*Danaus plexippus*), to evaluate whether neonicotinoids present a threat to the protection and conservation of species at risk.

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