Neonicotinoids in Connecticut Waters

Surface Water, Groundwater, and Threats to Aquatic Ecosystems

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Executive Summary

Neonicotinoid insecticide use has increased markedly nationwide and in Connecticut during the 21st Century. Peer-reviewed studies and the US Environmental Protection Agency (USEPA) link neonicotinoids to devastating declines in birds, bees, butterflies, and other insects, and to the jeopardizing of over 200 endangered and listed species. The high solubility of neonicotinoids in water makes them a potential threat to aquatic ecosystems, in particular to aquatic macroinvertebrates, as well as to the fish, frogs, birds and other wildlife that depend on them for survival. Water quality monitoring by the US Geological Survey (USGS) shows that neonicotinoids frequently and consistently appear in Connecticut's surface waters at levels expected to cause significant harm to the state's aquatic ecosystems and which also represent the potential for human health harms.

Estimating the amount of pesticide used in Connecticut remains a challenge. Imidacloprid, one of the earliest and most widely used neonicotinoids, was detected in 45% of surface water samples tested between 2001 and 2024, and was detected in 11% of groundwater samples tested between 2002 and 2017. All positive tests of imidacloprid in Connecticut represent levels above the USEPA chronic benchmark for aquatic invertebrates, which is the concentration that is expected to cause harm during prolonged exposure. Imidacloprid has become more frequently detected in Connecticut surface water through time, whereas the frequency of imidacloprid detection in groundwater did not increase through time. Seasonal patterns in imidacloprid detection reflect greater spring and summer applications of neonicotinoids for agricultural pest control or for the care of manicured lawns and golf courses, but they also show levels consistently above the chronic benchmark for every month in which they were detected. Chronic year-round exposure indicates continual stress to aquatic insects at all life stages.

Imidacloprid concentration has been increasing through time in Connecticut surface waters. The highest concentrations (eight times higher than the USEPA chronic benchmark for aquatic life) were detected in the only targeted study in Connecticut, which was designed to sample when (summer) and where (near large expanses of manicured turf grass) neonicotinoids are typically used for pest control in suburban settings. These results suggest that targeted sampling of areas (e.g., waters near row crops such as corn and soybeans, near golf courses, or near suburban areas with manicured lawns) during the summer months is more likely to reflect the presence of neonicotinoids than do the data currently provided by the USGS. In addition, imidacloprid concentrations increase toward southern Connecticut, possibly indicating greater use in the southern parts of the state or the movement of imidacloprid south through streams and rivers. Nonetheless, surface waters throughout most of the state remain untested for neonicotinoids.

The effects of imidacloprid on biota remain poorly understood in Connecticut because of the absence of studies that test for neonicotinoids and that survey macroinvertebrate communities at the same time and in the same places. However, evidence from the Norwalk River implies a possible decline in the abundance and richness of some ecologically important species, such as mayflies, which serve as a key food source for fish and other macroinvertebrates and help recycle nutrients in the water column. The potential impacts of neonicotinoids on biodiversity throughout the state warrants critical investigation.

Testing of groundwater for neonicotinoids has been sporadic (mostly restricted to 2003 and 2017) and does not provide sufficient information to adequately assess the persistence or

occurrence of neonicotinoids in groundwater, which is concerning in a state where so many residents depend on well water. To understand the frequency with which imidacloprid infiltrates groundwater, representing a potential threat to human health, a protocol must be established for more consistent sampling and testing of groundwaters. More intensive monitoring of both surface and ground waters is needed in Connecticut, especially for neonicotinoid compounds that are not currently monitored by the USGS, including acetamiprid, clothianidin, and thiamethoxam.

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1. Introduction

1.1 Neonicotinoids: Their Use and Environmental Concerns

Neonicotinoids (also known as neonics) include a variety of chemical variants (e.g., nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid, and thiacloprid) and are now the most widely used class of insecticides in the world, having quickly grown in popularity since they became commercially available in the 1990s (Goulson, 2013). Neonicotinoids are nicotinic acetylcholine receptor agonists, binding strongly to nicotinic acetylcholine receptors in the central nervous system of insects. Although these pesticides cause nervous stimulation at low concentrations, higher concentrations can induce receptor blockage, paralysis, and death (Goulson, 2013). Very small amounts of neonicotinoids are harmful to insects. One square foot of lawn treated with a neonicotinoid pesticide at EPA-approved levels can contain enough of the chemical to kill over a million bees (NRDC). The oral LD50 (lethal dose that kills 50% of a population) of clothianidin is 3.8 ug for a bee (European Commission 2005). Consequently, a coating on each seed contains enough neonicotinoids (~1.25 milligrams = 1,250,000 ng) to kill over 150,000 bees. Neonicotinoids are about 10,000 times more toxic (Suchail et al., 2000) to insects than is dichlorodiphenyltrichloroethane (DDT). Because neonicotinoids bind more strongly to the receptors of insects than to those of vertebrates, they were considered to be safer to humans than the insecticides that they replaced (e.g., organophosphates, carbamates, and pyrethroids). However, a growing body of research links neonicotinoids to a range of human health harms (e.g., Cimino et al., 2017).

The Ecological Risk Assessment Process conducted by the US Environmental Protection Agency (USEPA) for individual pesticides provides an understanding of the ecotoxicity of those pesticides and develops Aquatic Life Benchmarks. For the neonicotinoid insecticide imidacloprid, the USEPA chronic and acute benchmarks for fresh water are 0.01 ug/L (micrograms per liter) and 0.385 ug/L, respectively. Comparing a measured concentration of a pesticide in water with an Aquatic Life Benchmark can facilitate the interpretation of monitoring data and the identification and prioritization of sites and pesticides that may require further investigation. Importantly, the reliable detection limit for tests of imidacloprid is 0.016 ug/L. Consequently, all detections of imidacloprid in freshwater samples represent concentrations that are above the EPA chronic benchmark (i.e., 0.01 ug/L). Research indicates that negative impacts to invertebrates are occurring at the lowest detected concentrations when sustained over a long period of time (Van Dijk et al., 2013), which is alarming considering the near constant year-round sublethal concentrations observed in Connecticut rivers.

Because neonicotinoids are water soluble, readily absorbed by plants, and easily transported throughout the tissues of the plant, they provide protection against many forms of plant pests, including boring and root-feeding insects, which cannot be easily controlled via foliar sprays of other insecticides. The combination of water solubility and high toxicity to insects stimulated the prophylactic use of neonicotinoids as seed dressings, as they require no action from end users, but provide potential protection for all parts of the plant for several months after sowing (Jeschke et al., 2011). Although seed dressings account for most of their use, neonicotinoids are also commonly used on manicured lawns and turf grass, as foliar sprays for horticultural crops, garden sprays for flowers or vegetables, cockroach control, termite control, and topical application on pets to protect them from ectoparasite infestations (Armbrust and Peeler, 2002; Oliver et al., 2010; Jeschke et al., 2011).

The systemic and long-term persistence of neonicotinoids in all plant tissues, including pollen and nectar, can result in severe declines in non-target insect species (the taxon most sensitive to neonicotinoids) such as bees and butterflies. Many recent studies suggest that the broad application of neonicotinoids has contributed to the widespread declines in insect abundance and diversity, including the decimation of bee populations (Sanchez-Bayo and Wyckhuys, 2019). Indeed, the use of neonicotinoids may have effects opposite of those intended when used for large-scale agricultural practices. By decimating local populations of pollinators (Douglas et al., 2020) on which farmers rely to pollinate their crops, neonicotinoids can decrease rather than increase yield (Douglas et al., 2015).

For turf grass management, applications of neonicotinoids also did not reduce pest larvae in comparison to control plots (Clavet et al., 2014). Continued use may result in increased risk of insecticide resistance, disruption of biological control, risks to human health, and widespread negative effects on non-target species (e.g., bees, butterflies, aquatic invertebrates), all for little benefit to turf grasses.

Neonicotinoids can persist in the environment for considerable time depending on local environmental conditions. Imidacloprid, thiamethoxam, and clothianidin readily undergo direct photolysis in surface waters (half-lives of 0.20-3.3 days); however, light attenuation in depths as shallow as a few inches combined with water turbidity can result in longer persistence of neonicotinoids (Lu et al., 2015). When not directly exposed to sunlight, the half-life of neonicotinoids can be considerable. For example, imidacloprid has an estimated half-life of over 100 days in soil, allowing for season-long control of insects with a single application (Anhalt et al., 2007). Moreover, a single application, when injected into trees, can control termites for several years (Oliver et al., 2010). The combination of purported low toxicity to vertebrates (including humans), high toxicity to insects, flexible application, and environmental persistence resulted in neonicotinoids quickly becoming the most popular class of insecticide throughout the world. Nonetheless, three neonicotinoids were partially banned in the European Union in 2013 and in 2018 all outdoor use was banned to protect pollinators (e.g., bees) from precipitous declines in abundance. In 2019, Quebec banned the use of neonicotinoid-treated corn and soybean seeds without verification of need and permission for use. More recently, New York and Vermont have passed laws with similar restrictions that go into effect in 2029. New York, New

Jersey, Maine and Nevada bar the use of neonicotinoids on turf grass and ornamental landscaping.

Integrated pest management was developed shortly after World War II when it was recognized that pesticide application negatively affected non-target species, including populations of insect pollinators and natural enemies that serve to support crop production (e.g., Smith and Smith, 1949; Acosta, 1995-2006). Integrated pest management is based on the principles of (1) acceptable pest levels (rather than eradication), (2) preventative cultural practices, (3) regular monitoring of pest levels, (4) use of mechanical controls (e.g., traps, barriers), (5) use of biological controls (e.g., insects that parasitize or consume pest species), and (6) responsible use of pesticides only when absolutely necessary and at times of maximum effect (e.g., during stages of a pest's life cycle during which they are most vulnerable). In general, integrated pest management has helped to mitigate the effects of pesticides on non-target species (including humans), by restricting their use to situations in which they are deemed critical. However, the wide-spread use of neonicotinoids as seed dressings marked a dramatic departure from integrated pest management, an approach predicated on minimizing the use of chemical pesticides in favor of pest monitoring and pesticide application only when necessary (Metcalf and Luckmann, 1994). The abandonment of integrated pest management practices in the use of neonicotinoids has coincided with and been implicated in the decline of many non-target species of insects, in particular pollinators such as bees (e.g., Cresswell et al., 2012; van der Sluijs et al., 2013; Lundin et al., 2015; Woodcock et al., 2016) and monarch butterflies (Van Deynze et al., 2024). Because many species feed on insects (e.g., other insects, fish, frogs, toads, lizards, small mammals, birds, bats), insects often represent a foundational component of food webs (Frank and Tooker, 2020). Consequently, drastic declines in insect abundance can create a trophic cascade in which species that depend on them for food experience concomitant declines in abundance (e.g., Bowler et al., 2019; Tallamy and Shriver, 2021; Rochlitz et al., 2024).

In addition to well documented threats to non-target terrestrial organisms, the high solubility of neonicotinoids in water makes them a potential threat to aquatic ecosystems as well, in particular to aquatic macroinvertebrates because insects are the most sensitive taxon to neonicotinoids. Because neonicotinoids were first used heavily in agricultural areas (e.g., USGS, 2024), concerns about the effects of neonicotinoids on aquatic systems first arose in the mid-western US, where neonicotinoid use as prophylactic seed dressing was ubiquitous by 2009. Neonicotinoid concentrations in tributaries of the Great Lake have been up to 40 times greater than the USEPA chronic benchmark (Hladik et al., 2018). Mayflies, in particular *Hexagenia* spp., are among the most sensitive aquatic insects to neonicotinoids. Even at sublethal levels, the presence of neonicotinoids leads to greater susceptibility to hypoxia, reduced fitness, and increased predation in mayflies (Bartlett et al., 2018). Worrying declines in mayfly abundance across the US are being documented (e.g., Stepanian et al., 2020); however, identifying the relative contributions to these declines that are associated with the many pesticides used commercially is challenging. The USGS conducted five Regional Stream-Quality Assessment studies to assess stressor effects on stream ecology, which implicated various pesticides as likely stressors that adversely affect aquatic invertebrate communities (Van Dijk et al., 2013; Nowell et al., 2024). Multiple modeling approaches identified the neonicotinoid imidacloprid (as well as bifenthrin, chlordane, and fipronil) as an important factor in explaining variation in aquatic invertebrate health among streams in the Northeastern US, with imidacloprid often exceeding USEPA chronic benchmarks for aquatic life (i.e., levels known to be toxic to aquatic invertebrates).

Despite historically being considered a relatively safe option for pest control, more recent studies have linked neonicotinoids to human health threats, including harms to heart and brain development in prenatally exposed children, (Cimino et al., 2017). In addition, laboratory studies on vertebrates show decreased sperm quality and quantity (e.g., Bal et al. 2012; Lonare et al. 2016; Mosbah et al., 2018), as well as decreased testosterone levels (Arican et al. 2020). The broad application of neonicotinoids has resulted in 63% of fruit and vegetable samples tested by the USDA containing at least one neonicotinoid, with 57% containing multiple neonicotinoids (Cimino et al., 2017). In addition, a Harvard study found at least one neonicotinoid was detected in all store-bought fruit and vegetable samples, with the exception of nectarines and tomatoes, and 90% of honey samples contained at least one neonicotinoid (Chen et al., 2014). Neonicotinoids are highly soluble in water, making them easily absorbed by plant tissues to provide protection against pests in the roots, stems, leaves, flowers, and fruits. Consequently, neonicotinoids cannot be washed off of food as they are contained within the tissues of fruits and vegetables. The CDC found at least one neonicotinoid in 49% of the US population in 2015-2016, with the highest concentrations found in children (Ospina et al., 2019). Even more concerning, a recent study (Buckley et al, 2022) found neonicotinoids in 95% of the pregnant women who participated in the study. The ubiquity of neonicotinoids in our food and environment has created an urgent need to understand potential short- and long-term effects on human health. A review of the effects of chronic exposure to neonicotinoids found appreciable risk for developmental and neurological harms to humans, including tetralogy of Fallot (a congenital heart condition), anencephaly (a fatal developmental condition), autism spectrum disorder, memory loss, and physical tremors (Cimino et al., 2017).

Although hundreds of peer-reviewed studies link neonicotinoid use to the collapse of populations of honeybees, butterflies, and other pollinators throughout the world (e.g., van der Sluijs et al., 2013; van Lexmond et al., 2015; Braak et al., 2018), the ubiquity of neonicotinoids and their potential impacts on aquatic environments have only recently been recognized and investigated. This report addresses potential issues in freshwater aquatic environments of Connecticut, including surface and ground waters, that may occur due to the prevalence of neonicotinoids.

We focus on imidacloprid for multiple reasons. First, it is the neonicotinoid that the USGS is evaluating in Connecticut surface and ground waters. Second, it was essentially the only neonicotinoid detected during the 2024 Clean Rivers Project conducted in southwestern Connecticut streams. Third, since their discovery in the late 1980s, neonicotinoids have become the most widely used class of insecticides worldwide, with imidacloprid being the single most commonly used insecticide in the world. Fourth, imidacloprid, as well as other neonicotinoids, are used widely in agriculture as well as to maintain turf grasses in residential areas and golf courses, in products for gardening (flowers, fruits, or vegetables), and as a pet treatment to prevent flea and tick infestations.

Aquatic ecosystems are critical to our economy and communities. They provide sources of drinking water, buffer communities against floods, and support sportfishing and recreational industries. Aquatic invertebrates are keystone species in river and stream ecosystems (Jacobus et al., 2019; Morse, 2009). Unfortunately, they are negatively affected by many environmental stressors (e.g., habitat degradation, increasing temperatures, nutrient enrichment), which potentially makes them more highly susceptible to the effects of pesticides used in terrestrial habitats that wash into streams and rivers.

The focus of this report is aquatic systems because multiple regulatory and non-regulatory assessments have shown that aquatic organisms may be exposed to imidacloprid, and aquatic invertebrates in particular are highly sensitive to imidacloprid exposure. The USEPA sets two freshwater aquatic invertebrate benchmarks for insecticides: acute and chronic concentrations (USEPA, 2024). Acute benchmarks estimate pesticide concentrations that are expected to cause harm during short-term exposure (from hours to days). In contrast, chronic benchmarks estimate pesticide concentrations that may cause harm during prolonged exposure (from weeks to months). These benchmarks are based on responses of the most sensitive species and represent values below which pesticides are not expected to represent risk for aquatic life (USEPA, 2024).

1.2 Goals

The general goals of this report are to evaluate the spatiotemporal distribution of neonicotinoids in Connecticut surface and ground water, and to assess their potential harmful effects on ecosystems. This will inform recommendations that lead to a better understanding of contemporary and future status of neonicotinoids in Connecticut and their potential deleterious effects on aquatic fauna. More specifically, our goals were five-fold:

(1) Determine long-term and seasonal patterns of the frequency of imidacloprid occurrence in surface and ground waters of Connecticut;
(2) Determine seasonal variation in imidacloprid concentration in Connecticut waters;
(3) Determine spatiotemporal variation in imidacloprid concentration in Connecticut surface water and groundwater;
(4) Leverage long-term sampling from a site in the Connecticut River from northern Connecticut (Thompsonville) as a case study to evaluate long-term patterns in imidacloprid concentration that reflect impacts from a “light urban” region that contains urban, forested, and agricultural areas in Massachusetts that flow south into Connecticut; and
(5) Use the Norwalk River, a watershed with relatively little agriculture, as a case study to evaluate long-term trends in imidacloprid concentration from non-agricultural outdoor sources, and long-term trends in aquatic macroinvertebrate richness and abundance.

2. Data

2.1 USGS Imidacloprid Data

To evaluate spatial and temporal patterns of neonicotinoids in Connecticut, we used all known results from water samples that have been tested for neonicotinoids in Connecticut. The majority of these data was collected from October of 2001 to January of 2024 by the United States Geological Survey (USGS), which tested a total of 662 water samples (600 surface water and 62 groundwater samples) from Connecticut for imidacloprid (Table 1).

Surface water samples were collected from 66 sites associated with 23 rivers and creeks in Connecticut (Table 1; Figure 1). The USGS first collected surface water samples to test for imidacloprid in 2001 and 2002, but regular testing of surface water for imidacloprid did not

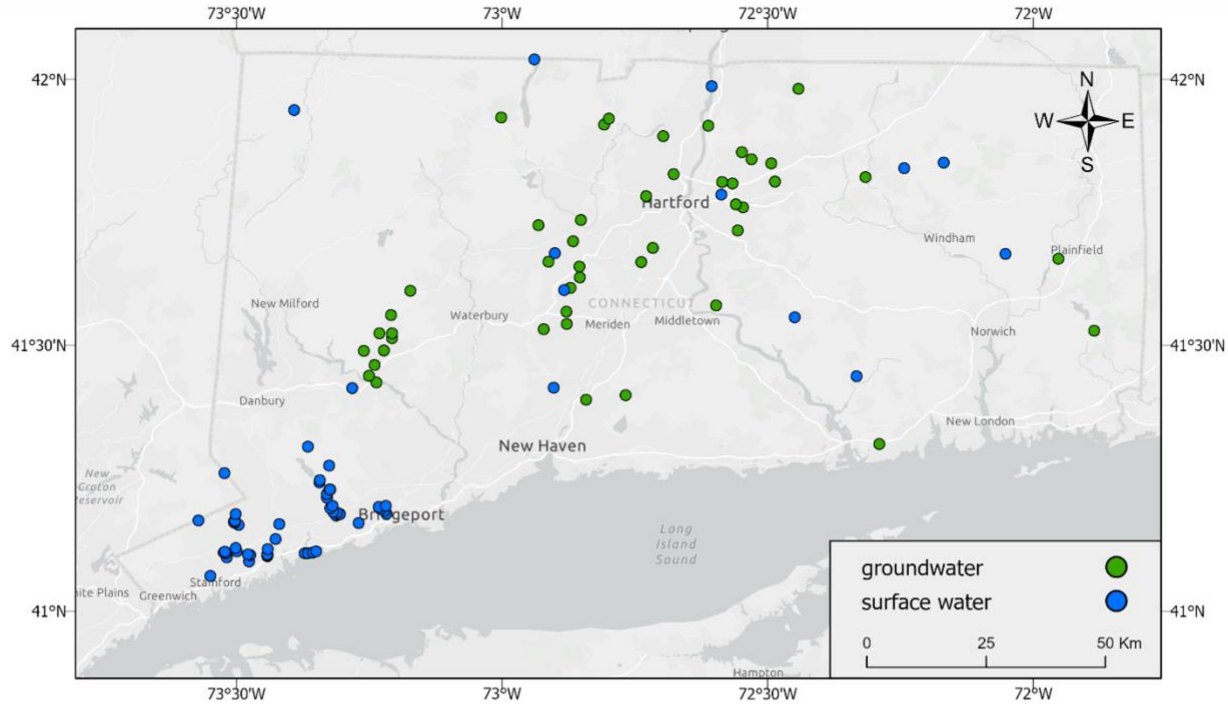

Figure 1. Locations of surface and ground water samples in Connecticut.

commence until December of 2009, with at least a dozen samples from surface water tested for imidacloprid every year (with the exception of 2011). Although the total number of surface water samples tested for imidacloprid is considerable (600), over 200 samples were from a single location on the Connecticut River (Thompsonville), and the majority of the remaining samples are from Fairfield County (Figure 1), demonstrating a need for more comprehensive testing throughout the state.

In contrast to the regular sampling of surface water, groundwater testing has been sporadic (Table 2), including only 62 samples from 46 wells throughout the state (Table 2; Figure 1). Because rural residents in Connecticut typically rely on private wells for residential water, the lack of knowledge of neonicotinoids in Connecticut groundwater represents a potential human health risk. Testing of groundwater for imidacloprid occurred between 2002 and 2004, and then again in 2017, with no recent groundwater testing of neonicotinoids from anywhere in the state. This has led to a large disparity in the number of samples tested for imidacloprid in surface versus ground waters (Table 2). In addition, the groundwater samples have come almost exclusively from the central part of the state, with effectively no testing of groundwater for any neonicotinoid in the northwestern, eastern, or coastal portions of the state. The lack of information about neonicotinoids in the groundwater of large swaths of suburban parts of the state, as well as the lack of any information about neonicotinoids other than imidacloprid in Connecticut groundwater, are concerning.

2.2 The Clean Rivers Project Neonicotinoid Data

During 2024, the non-profit, Pollinator Pathway, Inc., funded a small-scale study to investigate the presence of neonicotinoids in 10 streams and rivers in lower Fairfield County. The Clean Rivers Project was designed to detect and quantify the presence of six neonicotinoids (i.e., nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid, and thiacloprid) in surface

Table 1. Sources of water samples from Connecticut that were tested for imidacloprid.

Water source	Sites	Samples	Imidacloprid detected
Aspetuck River	5	5	1
Blackberry River	6	7	5
Connecticut Rive	1	202	39
Eightmile River	1	1	0
Fenton River	1	4	0
Five Mile River	7	7	6
Goodwives River	3	4	4
Hockanum River	1	9	9
Housatonic River	5	6	0
Hubbard River	1	4	0
Little River	1	1	0
Mill River	6	23	21
Mount Hope Rive	1	1	0
Noroton River	2	3	3
Norwalk River	2	307	163
Pequabuck River	1	9	9
Pootatuck River	1	9	6
Quinnipiac River	1	9	8
Rippowam River	1	9	7
Rooster River	6	15	15
Salmon Creek	1	4	0
Salmon River	1	4	0
Saugetuck River	11	13	2
Groundwater	46	62	7

waters adjacent to large expanses of manicured lawns such as golf courses, which commonly use neonicotinoids to control pests that damage turf. In total, 56 surface water samples were collected and tested for six neonicotinoids by analytical laboratories of the Center for Environmental Sciences & Engineering at the University of Connecticut. To our knowledge, these are the only samples from Connecticut that have been tested for neonicotinoids other than imidacloprid.

2.3 Connecticut Department of Energy and Environmental Protection Macroinvertebrate Data

We acquired data on macroinvertebrates that were collected from 17 different locations along the Norwalk River and its tributaries between 1989 and 2020. These samples were collected using standard CTDEEP protocols and a Rapid Bioassessment Protocol 3 level of effort (Plafkin et al., 1989). To avoid complications associated with seasonality, we only used samples collected during the fall, the time when 77 of the 81 samples were collected. Each macroinvertebrate sample was characterized by total abundance (the total number of individuals in the sample regardless of their taxonomic designation) and by richness (the number of distinct taxa identified in each sample). Because species-level identifications were not always possible, macroinvertebrates were identified to the lowest possible taxonomic level, usually to the level of genus or species. Consequently, when we refer to “richness” in this report, it is a general reference to taxonomic richness rather than to species richness. Nonetheless, all taxonomic designations were unique, thereby providing estimates representing the minimum number of species in each sample.

3. Statistical Analysis

Because imidacloprid may occur at levels below detection limits, we substituted a value of ½ of the detection limit for samples from which imidacloprid was not detected. This is an accepted procedure for estimating concentrations that may be below the ability of procedures or equipment to detect reliably (Beal, 2001; Noventa et al., 2024). The detection limit for imidacloprid has changed through time, being 0.007 ug/L from 2001-2004, raised to 0.02 ug/L from 2005-2006 (a time for which we have no data from Connecticut), then raised again during

2006 to 0.06 ug/L, until 2013, when the limit was lowered to 0.016, where it remains today (Table 2).

We used general linear models to determine if the frequency of imidacloprid detection exhibits a temporal trend, differs among water sources (surface vs. ground), or if temporal trends depend on water source. In addition, we conducted simple linear regressions to determine temporal trends in imidacloprid frequency separately for each water source.

Table 2. Data from Connecticut that was used to evaluate spatiotemporal dynamics of imidacloprid or attributes of macroinvertebrates from the Norwalk River. Concentrations are in ug/L (micrograms per liter).

Year	Surface water samples	Ground water samples	Macro-invertebrate samples	Mean imidacloprid concentration	USGS detection limit	Maximum imidacloprid concentration
1989	--	--	4	--	--	--
1997	--	--	9	--	--	--
1998	--	--	--	--	--	--
2000	--	--	1	--	--	--
2001	4	--	--	0.004	0.007	0.000
2002	25	7	1	0.005	0.007	0.034
2003	--	31	--	0.005	0.007	0.036
2004	--	8	--	0.006	0.020	0.000
2005	--	--	--	--	--	--
2006	--	--	7	--	--	--
2007	--	--	11	--	--	--
2008	--	--	--	--	--	--
2009	1	--	3	0.030	0.060	0.000
2010	17	--	4	0.032	0.060	0.063
2011	--	--	8	--	--	--
2012	12	--	--	0.052	0.060	0.216
2013	41	--	10	0.010	0.016	0.045
2014	42	1	--	0.010	0.016	0.036
2015	41	--	3	0.012	0.016	0.048
2016	142	--	4	0.038	0.016	0.567
2017	40	15	5	0.015	0.016	0.142
2018	42	--	--	0.019	0.016	0.157
2019	42	--	--	0.016	0.016	0.092
2020	32	--	7	0.017	0.016	0.052
2021	42	--	--	0.016	0.016	0.071
2022	40	--	--	0.013	0.016	0.139
2023	36	--	--	0.014	0.016	0.123
2024	57	--	--	0.084	0.016	0.868

3.1 Broad Scale Analysis: State of Connecticut

A general linear mixed-effects model (Stroup, 2012) was used to evaluate spatiotemporal dynamics in imidacloprid concentrations in Connecticut. More specifically, year, latitude, longitude, sample type, and interactions between sample type and each of three other characteristics (i.e., year, latitude, and longitude) were explanatory factors in a model explaining variation in imidacloprid concentration. Finally, a random factor of site was included to account for repeated measures from the same location. This model provided an evaluation of patterns in space and trends in time, as well as facilitated an assessment of whether those patterns differ between water sources.

3.2 Small Scale Analysis: Connecticut River

A general linear mixed-effects model was used to evaluate the effects of time, sample type, and their interaction on imidacloprid concentration in the Connecticut River. All surface water samples were collected from the same location in the Connecticut River (Thompsonville, USGS-01184000, 41.9873186 N, 72.6053669 W), whereas groundwater samples were collected from 16 different wells in the Connecticut River Basin. A random factor of site was included to account for repeated measures from the same location.

3.3 Case Study: Norwalk River

The Norwalk River system is the only place in Connecticut for which we have data on imidacloprid concentration as well as data on aquatic macroinvertebrate abundance and richness from surface waters. Unfortunately, no studies have been designed with the specific goal of evaluating the effects of neonicotinoids on aquatic macroinvertebrate communities in

Connecticut. Consequently, the data from the Norwalk River for aquatic macroinvertebrates and for imidacloprid concentration are not matched in time and space. This makes evaluation of the potential effects of imidacloprid on macroinvertebrates particularly challenging to establish and any conclusions from such analyses may be controvertible. Nonetheless, these remain the best data in Connecticut to use for a preliminary evaluation of potential effects of imidacloprid on aquatic fauna. Although we cannot directly evaluate effects of imidacloprid on aquatic macroinvertebrates, we can evaluate temporal trends in imidacloprid occurrence and concentration as well as temporal trends in macroinvertebrate abundance and richness. Results from such preliminary analyses can indicate trends that may be alarming or encouraging, and can form the basis on which to design studies targeted to answer the question, "What effects are neonicotinoids having on aquatic macroinvertebrates in suburban Connecticut?"

To this end, we conducted simple linear regression to characterize temporal trends in imidacloprid concentration, frequency of samples with imidacloprid concentration above the detection limit, macroinvertebrate abundance, macroinvertebrate richness, mayfly abundance, and mayfly richness. Mayflies were evaluated separately from the rest of the aquatic macroinvertebrate fauna because they are the most sensitive taxon to neonicotinoids.

3.4 Attribution of Significance

Regardless of analysis, we chose to ascribe statistical significance to P-values ≤ 0.10 for two reasons. First, we were concerned with the consequences of failing to detect patterns in imidacloprid use that warrant additional scrutiny. Second, the data used in this report were not collected for the express purpose of evaluating spatiotemporal dynamics in imidacloprid use or their effects on aquatic invertebrates, making sampling design less than ideal to evaluate these environmental questions. In this context, the use of a higher than the standard 0.05 designation for significance increases the ability to detect patterns that may be of environmental, ecological, or human health concern.

3.5 Statistical Programs

General linear mixed effects models were conducted using the lme function from the nlme package (Pinheiro et al., 2022). General linear models, including simple linear regression, were conducted using the lm function from the stats package (R Core Team, 2024). All analyses were executed in R version 4.3.1 (R Core Team 2024).

4. Imidacloprid in Connecticut

A total of 718 water samples from Connecticut have been tested for neonicotinoids; however, most of those samples (~92%) have only been tested for imidacloprid. Analyses of the 56 samples collected from streams and rivers adjacent to large expanses of manicured turf grass (such as near golf courses) in southwestern Connecticut that were tested for six neonicotinoids revealed considerable differences in the prevalence of different neonicotinoid compounds. Four neonicotinoids (i.e., acetamiprid, nitenpyram, thiacloprid, and thiamethoxam) were never detected in any samples, and clothianidin was detected in a single sample from the Noroton River in Darien. In contrast, imidacloprid was detected in 30 (54%) of those 56 samples. Once in the environment, thiamethoxam breaks down into clothianidin. In general, thiamethoxam is now the most common neonicotinoid used for agricultural purposes (Simon-Delso et al., 2014), whereas imidacloprid remains the most common neonicotinoid used in urban or suburban settings such as lawn or golf course care (Hladik and Kolpin, 2015).

The USEPA chronic freshwater macroinvertebrates benchmark for imidacloprid is 0.01 ug/L (USEPA, 2024). All detections of imidacloprid in this study represent levels above the chronic benchmark, indicating the potential for harm to aquatic life. In addition, imidacloprid concentrations were above the USEPA acute freshwater macroinvertebrates benchmark (0.385 ng/L) for three samples: one in 2016 from the Rooster River in Fairfield, and two samples in 2024 from the Good Wives River in Darien. Levels above the USEPA acute benchmark signal severe effects (e.g., death of half the individuals in an aquatic invertebrate population) from even short-term exposure.

Prior to 2017, the USEPA chronic benchmark for freshwater invertebrates was 1.05 µg/L (1,050 ng/L). This value was based on limited older data and was not reflective of aquatic invertebrate sensitivity. In 2017, the USEPA updated the chronic benchmark to 0.01 µg/L (10 ng/L) to better reflect the sensitivity of aquatic invertebrates, particularly mayflies and midges (which are orders of magnitude more sensitive to imidacloprid than are the standard aquatic test organism, *Daphnia magna*). This brought USEPA's chronic benchmark into closer alignment with European Food Safety Authority (EFSA), which also uses 0.01 µg/L as its chronic benchmark.

Importantly, the European Union has now taken an even more precautionary approach. Based on EFSA’s 2018 assessment, a more refined chronic threshold in the range of 5.7-6.8 ng/L (0.0057-0.0068 µg/L) has been adopted in the scientific literature. This lower range stems from chronic studies on highly sensitive species and application of safety factors (typically 10x safety factor applied to a “no observable effect” concentration) to account for uncertainties in real-world conditions. The 5.7-6.8 ng/L benchmark better captures the risks posed by chronic, low-level imidacloprid exposure to sensitive aquatic invertebrates.

In Connecticut, mean annual imidacloprid concentration ranged from a low of 0.04 ug/L (micrograms per liter) in 2001 to high of 0.084 ug/L in 2024. The annual maximum detected concentration of imidacloprid ranged from non-detection (i.e., < 0.016 ug/L) during the years in which few samples were collected (4 surface water samples in 2001, 8 ground water samples in 2004, and one surface water sample in 2009) to a high of 0.868 ug/L in 2024. Importantly, because neonicotinoids can break down quickly in the environment, primarily due to photolysis (the breakdown of chemical compounds when exposed to light), it can be challenging to accurately estimate maximum concentrations of neonicotinoids to which wildlife are exposed (e.g., concentrations close to points and times of pesticide application). In effect, one would have to know that neonicotinoids were going to be applied, and then local waters would need to be tested for neonicotinoids soon after, especially in the event of heavy rainfall, to determine acute exposure levels. Consequently, the reported mean and maximum concentrations represent underestimates of exposure to neonicotinoids in Connecticut waters. Unfortunately, the amount by which these concentrations are underestimated is not known.

4.1 Temporal Trends in the Frequency of Imidacloprid Detection

Long-term trends in the frequency of detection of imidacloprid in Connecticut waters were contingent on water source (Table 3), with the frequency of detection increasing through time in surface waters but showing no significant temporal trend in groundwaters (Figure 2).

Surface waters.—The frequency of imidacloprid detection in surface waters has increased greatly over the past 12 years (Figure 2). Prior to 2012, detection rates were never above 10% in surface waters; however, 46% of samples collected since 2012 have detected imidacloprid, with at least half of the samples testing positive for imidacloprid during 5 of the past 9 years (Figure 2). Importantly, with the exception of the sampling conducted for the Clean Rivers Project during 2024, sampling has not targeted times or locations in which imidacloprid use is expected to be likely. Imidacloprid use may have plateaued over the past decade, with it essentially being pervasive throughout the state at the present time. Nonetheless, surface waters throughout much of the state remain untested for imidacloprid (Figure 1). Indeed, even locations that have been tested previously have typically only been tested once or a few times (Table 1) since testing started in 2001 (Table 2), with many samples taken from months during which neonicotinoid use is expected to be low (i.e., October through April). The dearth of testing throughout most of the state represents a sizeable knowledge gap that must be addressed to understand the potential risk to humans and to wildlife from neonicotinoids in the environment.

Groundwaters.—In Connecticut, the testing of groundwater has been much less consistent through time than the testing of surface water, though with slightly better spatial coverage (Figure 1). Groundwater has been tested for imidacloprid during only 5 years, with 74% of groundwater samples tested during 2003 or 2017, and with no tests

Table 3. Analysis of covariance evaluating effects of water source (surface vs. groundwater), time (Year), and their interaction (Source x Year) on the proportion of samples with detectable levels of imidacloprid. The overall model was significant ($P < 0.001$) and explained 55% ($R^2 = 0.551$) of the variation in frequency of detectable imidacloprid concentrations in Connecticut. Temporal changes in frequency of imidacloprid detection differed between water sources, with frequency increasing over time in surface water, but showing no temporal trend in groundwater.

	df	SS	MS	F-value	P-value
Water source	1	0.232	0.232	10.393	0.005
Year	1	0.322	0.322	14.444	0.001
Source x Year	1	0.088	0.088	3.953	**0.062**
Residuals	18	0.401	0.022		

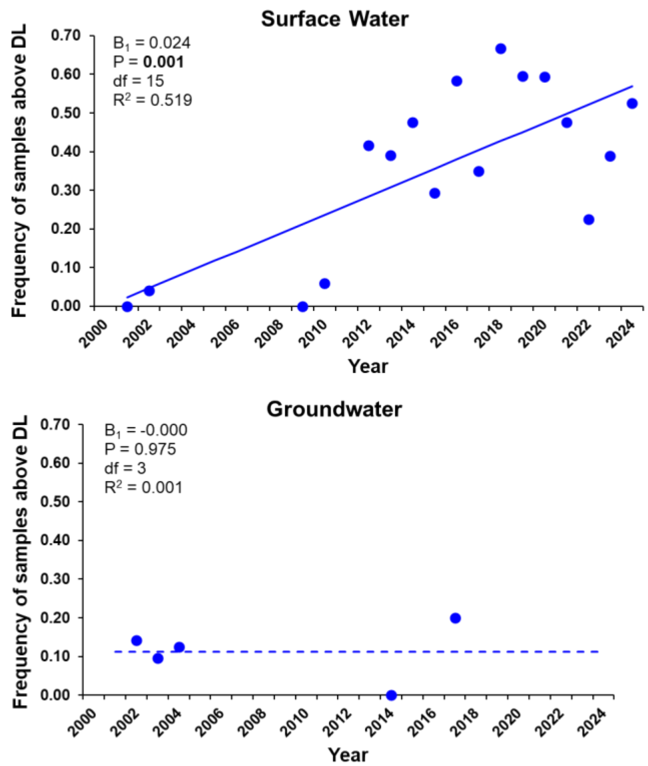

Figure 2. Temporal trends in the frequency of imidacloprid concentrations above the detection limit (DL) in Connecticut. Imidacloprid frequency in Connecticut surface waters has increased significantly through time, whereas frequency in groundwater has not changed significantly. Symbols indicate annual imidacloprid frequency of detection. Solid and dashed lines represent the best fit lines of significant and non-significant relationships, respectively. The slope (B_1), significance (P), degrees of freedom (df), and coefficient of determination (R^2) are from simple linear regressions.

of neonicotinoids from Connecticut groundwaters since then (Table 2). The lack of information about neonicotinoids in Connecticut groundwater makes detection of spatial or temporal trends challenging. To understand the frequency with which imidacloprid infiltrates groundwater, representing a potential threat to human health given the high number of residential wells that are in use in Connecticut, as well as how long it persists once in groundwater, a protocol must be established that more consistently samples and tests groundwaters throughout the state. The current groundwater data are not sufficient to draw strong conclusions. Interestingly, imidacloprid was found in groundwater more often than in surface water during the early years of sampling (2001-2004), whereas detection frequency in surface waters far surpassed that in groundwater in 2017. Nonetheless, the lack of groundwater data for the time period (2012-present) during which imidacloprid was more frequently found in surface waters, impedes the ability to understand the degree to which imidacloprid (or neonicotinoids in general) are a concern for Connecticut residents who use residential wells for drinking water. In contrast to its frequent occurrence in surface water, imidacloprid appeared in only 10-20% of ground water samples each year, with the exception of 2014, for which there was only one sample. Although imidacloprid is effective at infiltrating ground waters in sandy soil, the geology of Connecticut mitigates this potential issue in parts of the state where sandy soils are less pervasive.

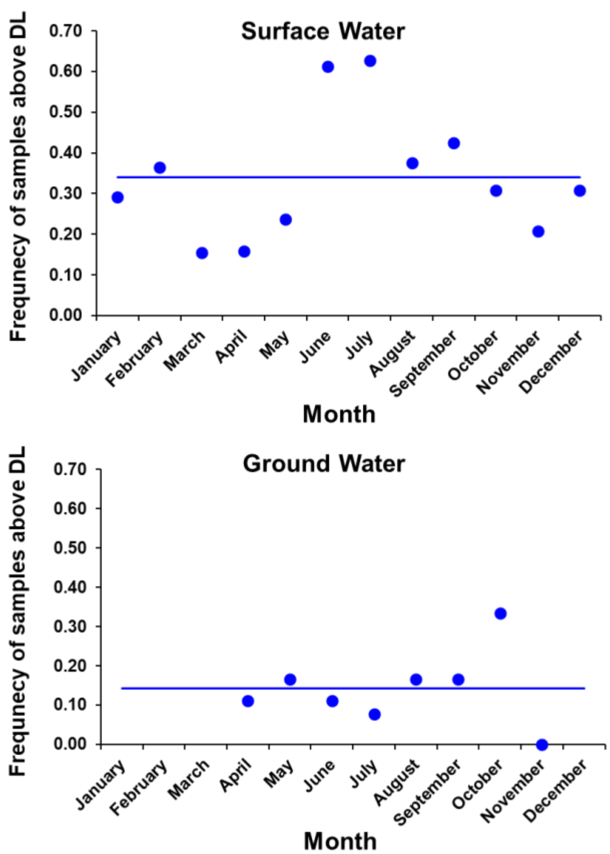

Figure 3. Seasonal trends in the frequency of imidacloprid concentrations above the detection limit (DL) in Connecticut. Monthly imidacloprid frequency in Connecticut surface and ground waters. Symbols indicate monthly frequencies, whereas lines indicate the overall average frequency.

An additional concern that applies to groundwater more than to surface water is the ability of neonicotinoids to persist for extended periods of time when not exposed to sunlight. Whereas the half-life of imidacloprid is a few hours to a few days in shallow surface waters (Lu et al., 2015), resulting in quick degradation and amelioration of potential chronic effects, imidacloprid can persist in shallow soils for over 100 days (Anhalt et al., 2007), and potentially much longer in deep drilled wells like those typically used in rural regions of Connecticut. However, without regular testing of groundwaters throughout the state, it will be difficult to know how pervasive or serious the risks are to humans from chronic exposure to neonicotinoids in drinking water. Once detected in a well, repeated testing is required to determine how long neonicotinoids persist. Moreover, once detected, remediation efforts can remove neonicotinoids via use of nanocomposite hydrogels (Alammar et al., 2020).

4.2 Seasonal Trends in the Frequency of Imidacloprid Detection and Concentration

Surface waters.—Seasonal patterns in imidacloprid detection and concentration in surface waters (Figures 3 and 4) reflect seasonal applications of neonicotinoids for agriculture or for the care of turf grasses (e.g., lawns and golf courses). In general, June, July, and August are the times of the greatest frequency and concentration of imidacloprid in surface waters, with average concentrations in June and July being six times greater than the USEPA freshwater aquatic chronic benchmark of 0.01 ug/L (Figure 3). Importantly, imidacloprid remains in surface waters throughout the year, with the average concentration from October through May remaining close to the USEPA chronic benchmark (Figure 4), indicating the potential for negative effects on aquatic invertebrates due to long-term, year-round, and potentially constant exposure.

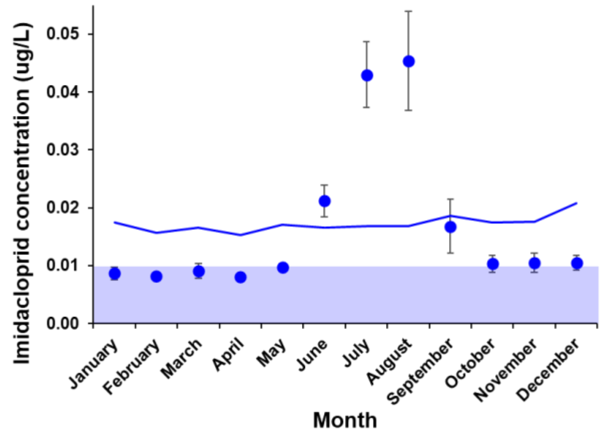

Figure 4. Seasonal trends in imidacloprid concentrations in Connecticut. Monthly imidacloprid concentration in Connecticut waters. Symbols indicate mean monthly concentrations (± 1 standard error). Blue line indicates the mean detection limit, which varies among months. Shaded blue area represents values below the USEPA chronic benchmark.

Groundwater.—In contrast to surface waters, groundwaters do not exhibit seasonal trends driven by recent imidacloprid applications (Figure 3). The slight increase noted for October represents a single positive in only three samples, which is not a sufficient sample size to determine if October represents an increase in imidacloprid frequency associated with summer-time applications. Groundwater sampling is not sufficient to confidently determine seasonal trends in imidacloprid occurrence or concentration in Connecticut groundwaters (Table 2), with no data collected during winter months.

4.3 Spatial and Temporal Patterns in Imidacloprid Concentration

Imidacloprid concentrations in Connecticut show two distinct trends (Table 4). First, imidacloprid concentrations have been increasing through time (Figure 5). Second, imidacloprid concentrations increase toward the coast (negative response to latitude). In contrast, there is no significant pattern of imidacloprid concentration going from east to west in the state, there are no significant differences between water sources (surface versus ground water), and there is no interaction between water source and time or between water source and space. Importantly, the dearth of groundwater testing makes drawing strong conclusions about differences in spatiotemporal patterns between water sources challenging. Nonetheless, including all of the available data in a single analysis is the most powerful way to determine if spatial or temporal

Table 4. Results (P-values) for analysis of the effects of time (year), geographic location (latitude, longitude), water source (surface vs. ground water), and interactions of water source with year, latitude, and longitude on concentration of imidacloprid in the State of Connecticut. Significant effects are bold, with blue indicating a positive effect (increasing) and red indicating a negative (decreasing) effect. Imidacloprid concentration has increased through time in Connecticut and is greater toward the coast than inland. Effects were consistent for surface and ground waters.

	Sample size	Year	Latitude	Longitude	Water source	Year x Source	Latitude x Source	Longitude x Source
Imidacloprid concentration	718	**0.028**	**< 0.001**	0.747	0.112	0.858	0.101	0.942

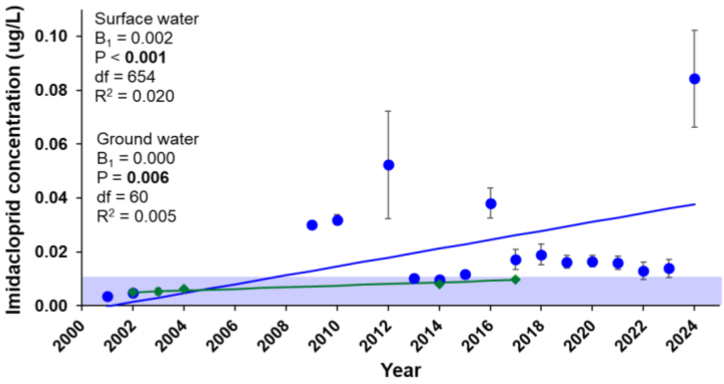

Figure 5. Temporal trends in imidacloprid concentration in surface and ground waters in Connecticut. Imidacloprid concentrations increased significantly in surface and in ground waters; however, the rate of increase was greater in surface waters. Blue symbols and line indicate mean monthly concentration (± 1 standard error) and best-fit regression line, respectively, for surface waters. Green symbols and line indicate mean monthly concentration (± 1 standard error) and best-fit regression line, respectively, for groundwaters. Shaded blue area represents values below the USEPA chronic benchmark. The slope (B_1), significance (P), degrees of freedom (df), and coefficient of determination (R^2) are from simple linear regressions.

patterns exist in imidacloprid concentrations in Connecticut.

Although imidacloprid concentration has increased through time in both surface and ground waters, the rate of increase was greater in surface waters than in groundwaters (Figure 5). Again, this comparison is hampered by the lack of more recent data for groundwater as well as the inconsistent sampling of ground water in general. Importantly, the frequency of imidacloprid detection in groundwater did not increase through time (Table 3; Figure 2). This apparent incongruency suggests that the current state of knowledge of neonicotinoids in Connecticut groundwater is insufficient to draw strong conclusions about imidacloprid occurrence in wells throughout the state or to effectively characterize potential negative effects on human health. This is especially concerning as only 16 groundwater samples have been tested for neonicotinoids during the past 20 years. Even more concerning, no groundwater samples have been tested during the past 7 years.

Compared to other years, 2024 exhibited the greatest average concentration of imidacloprid, with levels over three times that documented during the previous seven years. Importantly, the 2024 data from the Clean Rivers Project represent the only targeted sampling for neonicotinoids in Connecticut, with water samples taken specifically when (July and August) and where (near manicured turf grasses) one would expect to find high concentrations of neonicotinoids. This suggests that targeted sampling of areas (e.g., waters near row crops such as corn and soybeans, near golf courses, or near suburban areas with manicured lawns) during the summer months is more likely to reflect the current presence of neonicotinoids than are the data that are currently provided by the USGS.

The increasing concentrations of imidacloprid toward the coast (i.e., locations with lower latitude) could represent a combination of factors. First, as Connecticut rivers all eventually empty into Long Island Sound, imidacloprid in surface waters will move south from its location of application. Second, imidacloprid use is greatest in agricultural and suburban areas, and the proportion of area represented by the combination of agriculture and suburban developments increases toward the coast, with northern areas often being highly forested, which is a habitat type in which the use of neonicotinoids is uncommon (Armbrust and Peeler, 2002; Oliver et al., 2010; Jeschke et al., 2011). Importantly, ground water near the Connecticut coast in general, and in Fairfield County in particular, has rarely been tested for imidacloprid. Consequently, we have no information about the frequency or concentration of imidacloprid in groundwaters in the parts of Connecticut that have the greatest concentration of imidacloprid in surface waters.

4.4 Long-Term Trends in Imidacloprid Concentration in the Connecticut River Basin

Waters from the Connecticut River Basin exhibited contrasting patterns in imidacloprid concentration, with no significant temporal change in groundwaters, but with decreasing mean imidacloprid concentration in surface waters (Table 5; Figure 6). However, these results come with a number of caveats. First, water from wells in the Connecticut River Basin were only sampled during two years (i.e., 15 samples during 2003 and six samples during 2017). Samples from only 2 years are not sufficient to confidently evaluate temporal patterns. Second, the first year of sampling (2012) of surface water by the USGS from the station in Thompsonville drives the apparent decrease in imidacloprid concentration through time, with concentrations from 2013 through 2023 hovering around the USEPA chronic benchmark. Three of the nine samples from 2012 had concentrations > 0.135 ug/L. These samples likely captured high concentrations associated with runoff from a recent application of neonicotinoids before they degraded due to environmental exposure.

Table 5. Results (P-values) for analysis of the effects of time (year), water source (surface vs. ground water), and their interaction (Year x Source) on imidacloprid concentration in the Connecticut River Basin. Bold text indicates significant responses; however, because the temporal trend in imidacloprid concentration was contingent on sample type (decreasing through time for surface water and increasing through time for ground water), the direct effect of sample type cannot be interpreted.

	Sample size	Year	Water source	Year x source
Imidacloprid concentration	223	0.284	**0.046**	**0.078**

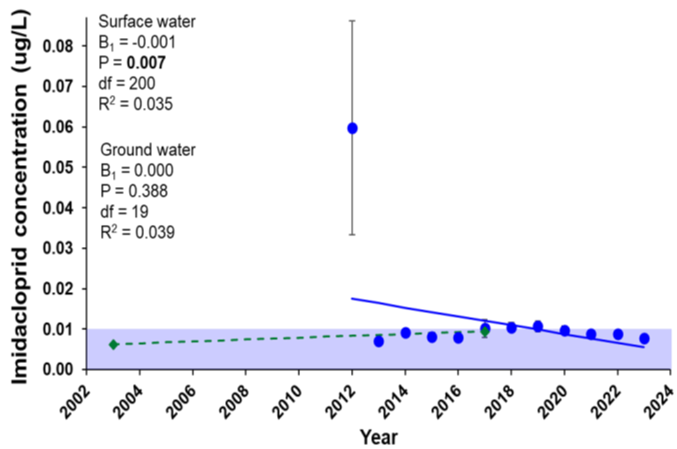

Figure 6. Temporal trends in imidacloprid concentration in surface and ground waters in the Connecticut River Basin. Imidacloprid concentrations decreased significantly in surface waters sampled in Thompsonville, CT, whereas concentrations in groundwaters did not exhibit a significant temporal trend. Blue symbols and solid line indicate mean monthly concentration (± 1 standard error) and best-fit regression line, respectively, for surface waters. Green symbols and line indicate mean monthly concentration (± 1 standard error) and best-fit regression line, respectively, for groundwaters. Shaded blue area represents values below the USEPA chronic benchmark. The slope (B_1), significance (P), degrees of freedom (df), and coefficient of determination (R^2) are from simple linear regressions. Solid and dashed lines represent significant and non-significant responses, respectively.

In contrast to the suburban Norwalk River area, the Thompsonville sampling location is classified as "light urban" because the waters reflect a combination of upstream urban, rural, and agricultural land uses in Massachusetts and northern Connecticut. Importantly, thiamethoxam has become the primary neonicotinoid associated with agricultural use rather than imidacloprid (Hladik and Kolpin, 2015), and these samples were only tested for imidacloprid rather than thiamethoxam or clothianidin (the neonicotinoid that results from the degradation of thiamethoxam). Consequently, the testing approach may underestimate neonicotinoids in this section of the Connecticut River because the neonicotinoids most likely to be used for agriculture are not being evaluated in water samples.

4.5 Norwalk River: a Case Study

Many scientific investigations have shown that neonicotinoid use results in the loss of invertebrate life in terrestrial and aquatic systems (e.g., Cresswell et al., 2012; van der Sluijs et al., 2013; Lundin et al., 2015; Woodcock et al., 2016; Bartlett et al., 2018; Stepanian et al., 2020; Nowell et al., 2024). Invertebrates are a critical link in food webs and their loss can lead to ecosystem-wide trophic cascades with losses of consumer species such as birds, fish, and mammals (Bowler et al., 2019; Frank and Tooker, 2020; Tallamy and Shriver, 2021; Rochlitz et al., 2024). Importantly, two groups that are extremely important for freshwater ecosystems in Connecticut are mayflies and caddisflies. With respect to neonicotinoids, mayflies are among the most sensitive aquatic invertebrates (Bartlett et al., 2018; Stepanian et al., 2020). The larval stages of these species feed on detritus, diatoms, and algae, making them important consumers in freshwater systems. In addition, various developmental stages of mayflies and caddisflies serve as an important resource for many vertebrate consumers; they are prey for fish, amphibians, reptiles, birds, and bats (Morse, 2009; Jacobus et al., 2019). Detected levels of imidacloprid alone (water samples have rarely been tested for other neonicotinoids) in Connecticut streams exceed levels (i.e., USEPA chronic benchmark for aquatic life) at which deleterious effects on stream invertebrates are expected to occur. The likelihood that neonicotinoids are causing ecosystem-wide damage in Connecticut is high. Substantial reductions in outdoor neonicotinoid use are required to mitigate further damage.

Data for imidacloprid from the Norwalk River exist from 2001 through 2024. In addition, data on aquatic macroinvertebrates from the Norwalk River exist from 1989 through 2020. These data are not from a study designed to determine the effects of imidacloprid on aquatic macroinvertebrates, and thus are not matched in time or space to facilitate an analysis of cause and effect. Nonetheless, these data represent the best available information to attempt to understand how temporal changes in neonicotinoids in Connecticut waters may affect the aquatic macroinvertebrates in those waters. Although we cannot determine direct responses to changes in imidacloprid

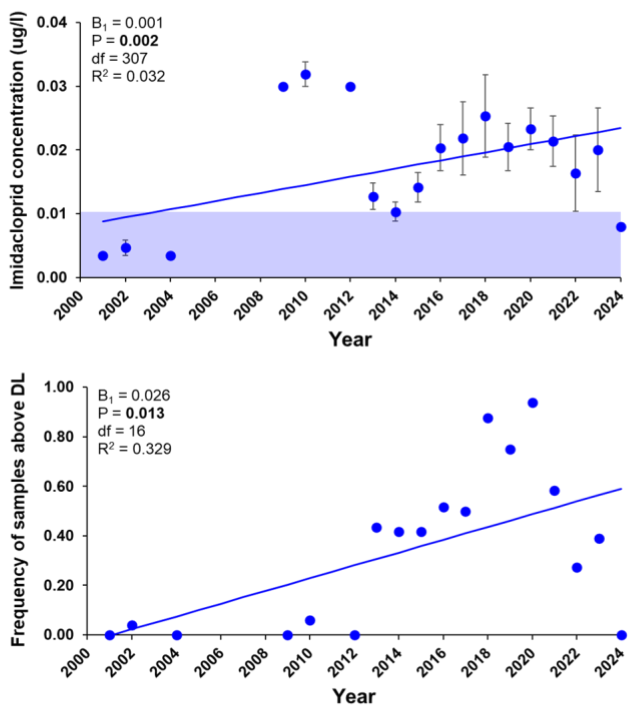

Figure 7. Temporal trends in imidacloprid concentration and in frequency of detection of imidacloprid in the Norwalk River.
Imidacloprid concentration and frequency of detection significantly increased through time. Blue symbols and lines indicate mean monthly concentration (± 1 standard error) and best-fit regression line or annual frequency of detection and best-fit regression line, respectively. Shaded blue area represents values below the USEPA chronic benchmark. The slope (B_1), significance (P), degrees of freedom (df), and coefficient of determination (R^2) are from respective simple linear regressions.

concentrations in the Norwalk River, we can (1) evaluate how imidacloprid concentrations have changed through time, (2) how aquatic macroinvertebrate abundance and richness have changed through time, and (3) determine if there is preliminary evidence that imidacloprid may be causing ecological harm to the aquatic macroinvertebrate community that requires a targeted study.

Imidacloprid concentration and frequency of detection have both increased through time in the Norwalk River (Figure 7). With the exception of 2024 (based on only one sample), mean imidacloprid concentration in the Norwalk River has been above the USEPA chronic benchmark every year since 2009. Imidacloprid was detected in over 40% of samples tested from the Norwalk River every year from 2013 through 2021 (Figure 7).

Macroinvertebrate abundance has not changed significantly through time in the Norwalk River; however, macroinvertebrate richness has significantly increased through time (Figure 8). In contrast, mayfly abundance and richness have significantly decreased through time. Mayfly abundance in 2020 was only one quarter of that observed in 1989, while mayfly richness was only one third of that observed in 1989 (Figure 8). For macroinvertebrate abundance to remain relatively stable and for macroinvertebrate richness to increase through time, other species of macroinvertebrate must have become more pervasive or more abundant to offset the losses associated with mayflies.

The increases in macroinvertebrate richness and abundance may be a consequence of the improvement in water quality of the Norwalk River, as water treatment plants along the river have been upgraded to reduce bacterial concentrations in the river. From 1998 through 2011, monitoring sites along the river consistently failed to meet water quality standards for

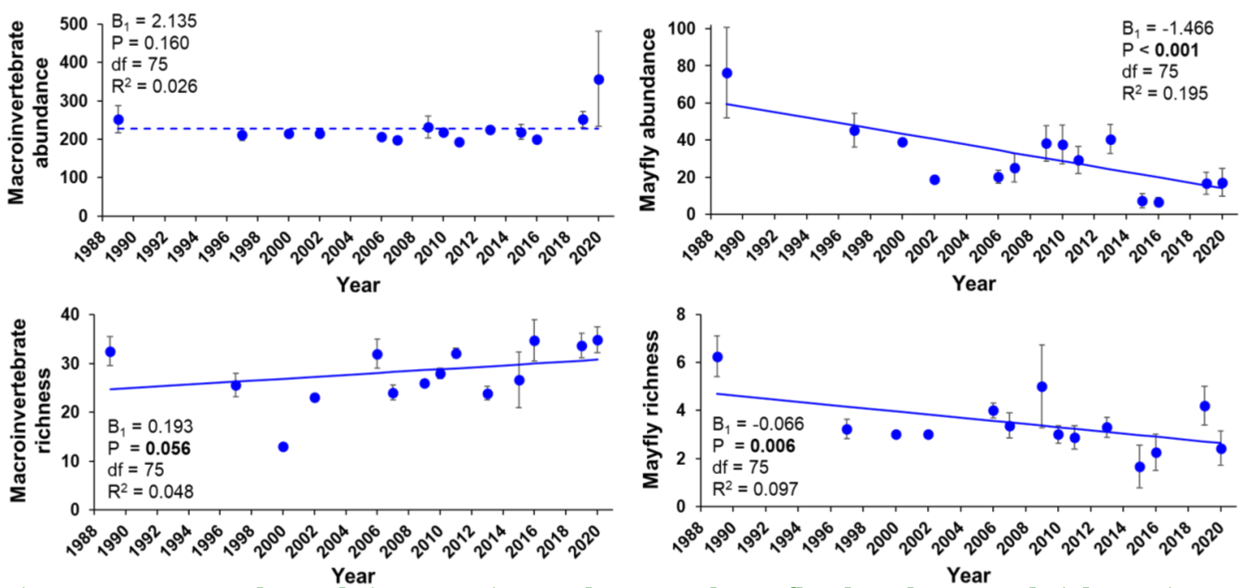

Figure 8. Temporal trends in macroinvertebrate and mayfly abundance and richness in the Norwalk River. Macroinvertebrate abundance does not exhibit a significant temporal trend, whereas macroinvertebrate richness significantly increased through time. In contrast, mayfly abundance and richness significantly decreased through time. Blue symbols and lines indicate mean monthly concentration (± 1 standard error) and best-fit regression lines, respectively. Solid lines and bold font indicate significant relationships, whereas dashed lines indicate a lack of a significant temporal trend. The slope (B_1), significance (P), degrees of freedom (df), and coefficient of determination (R^2) are from simple linear regressions.

recreational use, leading to this section of the river being added to the Connecticut Department of Energy and Environmental Protection list of impaired waters (Malik and Harris, 2014). This designation led to a concerted effort to improve water quality in the Norwalk River by implementing changes in septic system maintenance, lawn care, pet waste, and municipal stormwater management requirements. These activities reduced bacteria levels and improved water quality, (Spiller et al., 2022, 2023), except for the presence of "emerging contaminants" such imidacloprid, which has been increasing in frequency and concentration in the river over time (Figure 7). Improvements to water quality in the Norwalk River led to the removal of two sections of the river from the EPA impaired waterways list in 2012, but most locations on the river still fail to meet CTDEEP criteria for recreational use (e.g., Spiller et al., 2022, 2023). The Norwalk River is similar to many rivers in Connecticut in that is suffers from "urban stream syndrome", in which ecological degradation is driven by a complex array of pollutants largely delivered through urban stormwater runoff (Walsh et al., 2005).

Despite improvements in water quality in the Norwalk River, mayflies have decreased in abundance and richness (Figure 8). These declines may represent responses to increasing imidacloprid concentration, which are consistently above the USEPA chronic benchmark in the Norwalk River, indicating potential for harm to aquatic organisms. Because USEPA benchmarks are set based on the most sensitive species, such as mayflies, it is not surprising that mayflies are declining as imidacloprid concentrations are consistently above this benchmark. Nonetheless, a study designed specifically to evaluate aquatic macroinvertebrate responses to imidacloprid would be required to confidently ascribe causation to the decline of mayflies in the Norwalk River. Natural aquatic environments are dynamic systems subject to factors associated with human land use (e.g., nutrient enrichment from runoff; bacterial load associated with domestic animal waste or failing septic systems; industrial waste; and many dozens of pesticides used in agriculture, gardening, or lawn care) and represent a challenge for determining proximate causes to declines in populations. This highlights the importance of controlled experiments designed specifically to test responses of the biota to insecticides in natural environments (Schulz, 2004). To conduct robust studies that can confidently determine the proximate causes of changes in aquatic invertebrate populations and communities, studies are required that simultaneously characterize spatiotemporal patterns in environmental factors (e.g., water quality, including a broad spectrum of locally used insecticides) and in aquatic invertebrates (Nowell et al., 2024). Such studies can powerfully disentangle the complex interactions that may occur in dynamic situations in which water quality may be improving from some perspectives (e.g., reduced bacterial concentration, decreases in nutrient enrichment from local agriculture, restoration of riparian buffers; Malik and Harris, 2014), but may be declining from other perspectives (e.g., increases in neonicotinoids, algal blooms from increases in water temperature that facilitate the spread of *Didymosphenia* spp. and *Cymbella* spp.).

Importantly, documenting that pesticides are present in waters in which aquatic invertebrate communities are declining is not sufficient to conclude that pesticides are the proximate cause of declines in invertebrate populations or communities (Nowell et al., 2024). Multiple forms of evidence are required to determine the likelihood that pesticides are negatively impacting aquatic invertebrates, including toxicity predictions based on measured pesticide concentrations; statistical analyses that evaluate the relationships between pesticides and invertebrate populations and communities; multivariate models to identify which pesticides best explain variation in invertebrate populations, biodiversity, and composition; and controlled laboratory experiments to demonstrate the impacts of pesticides on aquatic invertebrates. Some of this information is already available. For example, toxicity predictions were evaluated by the USGS in over 400 streams and identified imidacloprid as a pesticide that very closely matched expectations: a prediction of 34% of streams with concentrations sufficient to cause aquatic invertebrate toxicity and a detection of toxic levels in 32% of streams (Nowell et al., 2024). In addition, imidacloprid was the candidate pesticide most often responsible for toxic levels (i.e., in exceedance of the USEPA chronic benchmark) and represented an astonishing 81% of all chronic benchmark exceedances throughout the U.S. In addition, many controlled laboratory experiments (e.g., Barlett et al., 2018; Schmidt et al., 2022) have tested the impacts of neonicotinoids on aquatic invertebrates. The final remaining piece required to understand the impacts of neonicotinoids on the fauna of Connecticut streams is to conduct studies that are capable of evaluating the relationships between pesticide concentrations and aquatic invertebrate communities in state waters.

5. Conclusions

Considerable research in North America and Europe has demonstrated that the use of neonicotinoids is associated with reductions in the abundance and diversity of invertebrate species in terrestrial and aquatic ecosystems. These effects can cascade throughout food webs and affect the abundance of consumer species such as birds, fish, mammals, and other vertebrates, potentially compromising the delivery of ecosystem services.

Detected levels of imidacloprid in Connecticut streams exceed levels at which deleterious effects on aquatic biota were observed in research elsewhere. In surface waters of Connecticut, the highest frequency of occurrence of samples above detection limit occurs during June and July. Similarly, the highest concentrations of imidacloprid occur during July and August. This reflects the seasonal use of pesticides for use on crops and manicured lawns, and corresponds to the reproductive periods of both invertebrate and vertebrate wildlife.

In general, the concentration of imidacloprid in Connecticut has increased significantly in surface waters (2001 to 2024) as well as in groundwater (2002 to 2017), signaling the more pervasive use of this chemical as the biocide of choice for controlling plant pests throughout the state. Moreover, the concentration of imidacloprid in Connecticut rivers increases in a southerly direction, being greatest toward the coast.

In contrast, the concentration of imidacloprid in the Connecticut River Basin, shows a complex pattern: significantly decreasing in surface waters (2012 to 2023) but not evincing a temporal trend in ground water (2003 to 2017). This phenomenon may arise as an artifact of sampling as all the temporal domain of sampling is quite different for the two water sources, and the detected decline in surface waters is strongly influenced by the exceptionally high concentration in 2012, with concentrations for all other years hovering at the USEPA chronic benchmark.

In surface waters of the Norwalk River, the frequency of occurrence of samples with concentrations of imidacloprid that are above detection limit as well as the concentration of imidacloprid has increased significantly from 2001 to 2024, with samples from the last decade consistently being above the USEPA chronic benchmark. During the same time period, the abundance and richness of mayflies, the insect taxon most sensitive to neonicotinoids, have decreased significantly.

Because the availability of data on neonicotinoids in Connecticut is essentially limited to imidacloprid, and because those data are constrained from both spatial and temporal perspectives, general conclusions are provisional at this time. Nonetheless, long-term trends concerning imidacloprid in surface waters are concerning and support more careful investigation based on rigorous principles of statistical design. Moreover, consequences of the effect of imidacloprid on biota remain poorly understood in Connecticut. Nonetheless, evidence from the Norwalk River implicates a possible decline in the abundance and richness of some ecologically important species (mayflies) and warrants critical investigation throughout the state.

6. Recommendations

Hereafter, we suggest a number of critical steps that should be considered to enhance understanding of spatial and temporal variation in neonicotinoid concentrations and their effects on macroinvertebrates in the State of Connecticut.

- Execute synoptic sampling (coordinated sampling in space and time) of neonicotinoid concentrations and macroinvertebrate abundance and richness.
- Expand the geographic sampling to include little studied areas of Connecticut (e.g., northwestern and eastern portions of the state).
- Increase the testing of ground water and well water for neonicotinoids, as these water sources are under-represented in the available data and may relate more intimately to human health concerns.
- Amplify testing to include samples of sediment, which may represent areas of contaminant accumulation and exposure for some benthic species.
- Enlarge the suite of neonicotinoids whose concentrations are being monitored throughout the state, including newer generation compounds such as cycloxaprid, imidaclothiz, paichongding, sulfoxaflor, guadipyr, and flupyradifurone.
- Implement before and after studies that focus on known pesticide application periods and major rainfall events to gather data that are relevant to possible acute levels of neonicotinoids.
- Explore the extent of sub-lethal effects of neonicotinoids on insects that include characteristics related to demographics such as emergence times, size at emergence, and proportion of individuals that reach maturity.
- Consider banning the use of seeds treated with neonicotinoids.
- Recommend the use of alternatives to neonicotinoids, including biological control and natural products, where feasible.
- Where non-toxic alternatives are not feasible, recommend the use of non-neonicotinoid insecticides such as chlorantraniliprole, which have low toxicity to bees, though they are toxic to aquatic invertebrates and butterflies.

- Conduct testing of effects of neonicotinoids on aquatic larvae in areas that are used for shellfish production. Many shellfish producers seed their oysters in the brackish areas near the mouth of large rivers, including the Quinnipiac River.

7. References

Acosta, E.W. 1995-2006. The history of integrated pest management (IPM). BioControl Reference Center. https://web.archive.org/web/20080807131454/http://www.biconet.com/reference/IPMhistory.html Accessed 4 December 2024.

Alammar, A., Park, S.-H., Ibrahim, I., Arun, D., Holtzl, T., Dumée, L., Lim, H.G., and Szekely, G. 2020. Architecting neonicotinoid-scavenging nanocomposite hydrogels for environmental remediation. Applied Materials Today 21: 100878.

Arıcan, E.Y., Kayali, D.G., Karaca, B.U., Boran, T., Öztürk, O., Okyar, A., Ercan, F., and Özhan, G. 2020. Reproductive effects of subchronic exposure to acetamiprid in male rats. Scientific Reports 10: 8985.

Anhalt, J.C., Moorman, T.B., and Koskinen, W.C. 2007. Biodegradation of imidacloprid by an isolate soil microorganism. Journal of Environmental Science and Health, Part B 42: 509-514.

Armbrust, K.L., and Peeler, H.B. 2002. Effects of formulation on the run-off of imidacloprid from turf. Pest Management Science 58: 702-706.

Bal, R., Naziroğlu, M., Türk, G., Yilmaz, O., Kuloğlu, T., Etem, E., and Baydas, G. 2012. Insecticide imidacloprid induces morphological and DNA damage through oxidative toxicity on the reproductive organs of developing male rats. Cell Biochemistry and Function 30: 492-499.

Bartlett, A.J., Hedges, A.M., Intini, K.D., Brown, L.R., Maisonneuve, F.J., Robinson, S.A., Gillis, P.L., and de Solla, S.R. 2018. Lethal and sublethal toxicity of neonicotinoid and butanolide insecticides to the mayfly, *Hexagenia* spp. Environmental Pollution 238: 63-75.

Beal, S.L. 2001. Ways to fit a PK model with some data below the quantification limit. Journal of Pharmacokinetics and Pharmacodynamics 28: 481-504.

Bowler, D.E., Heldbjerg, H., Fox, A.D., de Jong, M., and Böhning-Gaese, K. 2019. Long-term declines of European insectivorous bird populations and potential causes. Conservation Biology 33: 1120-1130.

Braak, N., Neve, R., Jones, A.K., Gibbs, M., and Breuker, C.J. 2018. The effects of insecticides on butterflies – a review. Environmental Pollution 242: 507-518.

Buckley, J.P., Kuiper, J.R., Bennett, D.H., Barrett, E.S., Bastain, T., Breton, C.V., Chinthakindi, S., Dunlop, A.L., Farzan, S.F., Herbstman, J.B., Karagas, M.R., Carmen J. Marsit, C.J., Meeker, J.D., Morello-Frosch, R., O'Connor, T.G., Romano, M.E., Schantz, S., and Schmidt, R.J. 2022. Exposure to contemporary and emerging chemicals in commerce among pregnant women in the United States: the environmental influences on Child Health Outcome (ECHO) Program. Environmental Science & Technology 56: 6560-6573.

Chen, M., Tao, L., McLean, J., and Lu, C. 2014. Quantitative analysis of neonicotinoid insecticide residues in foods: implication for dietary exposures. Journal of Agricultural and Food Chemistry 62: 6082-6090.

Cimino, A.M., Boyles, A.L., Thayer, K.A., and Perry, M.J. 2016. Effects of neonicotinoid pesticide exposure on human health: a systematic review. Environmental Health Perspectives 125: 155-162.

Clavet, C., Requintina, M., Hampton, E., Cowles, R.C., Byrne, F.J., and Alm, S.R. 2014. Clothianidin and imidacloprid residues in *Poa annua* (Poales: Poaceae) and their effects on *Listronotus maculicollis* (Coleoptera: Curculionidae). Journal of Economic Entomology 107: 2095-2102.

Cresswell, J.E., Desneux, N., and vanEngelsdorp, D. 2012. Dietary traces of neonicotinoid pesticides as a cause of population declines in honey bees: an evaluation by Hill's epidemiological criteria. Pest Management Science 68: 819-827.

Douglas, M.R., Rohr, J.R., and Tooker, J.F. 2015. Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield. Journal of Applied Ecology 52: 250-260.

Douglas, M.R., Sponsler, D.B., Lonsdorf, E.V., and Grozinger, C.M. 2020. County-level analysis reveals a rapidly shifting landscape of insecticide hazard to honey bees (*Apis mellifera*) on US farmland. Scientific Reports 10: 797.

European Commission. 2005. Chemicals, contaminants and pesticides: clothianidin, D.D.-F.S.P.a.d.c. Health & Consumer Protection Directorate-General, Brussels, Belgium.

Frank, S.D., and Tooker, J.F. 2020. Neonicotinoids pose undocumented threats to food webs. Proceedings of the National Academy of Sciences 117: 22609-22613.

Goulson, D. 2013. Review: an overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology 50: 977-87.

Hladik, M.L., and Kolpin, D.W. 2015. First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. Environmental Chemistry 13: 12-20.

Hladik, M.L., Corsi, S.R., Kolpin, D.W., Baldwin, A.K., Blackwell, B.R., and Cavallin, J.E. 2018. Year-round presence of neonicotinoid insecticides in tributaries to the Great Lakes, USA. Environmental Pollution 235: 1022-1029.

Jacobus, L.M., Macadam, C.R., and Sartori, M. 2019. Mayflies (Ephemeroptera) and their contributions to ecosystem services. Insects 10: 170.

Jeschke, P., Nauen, R., Schindler, M., and Elbert, A. 2011. Overview of the status and global strategy for neonicotinoids. Journal of Agricultural and Food Chemistry 59: 2897-2908.

Jetz, W., Wilcove, D.S., and Dobson, A.P. 2007. Projected impacts of climate and land-use change on the global diversity of birds. PLoS Biology 5: e157.

Lonare, M., Kumar, M., Raut, S., More, A., Doltade, S., Badgujar, P., and Telang, A. 2016. Evaluation of ameliorative effect of curcumin on imidacloprid-induced male reproductive toxicity in wistar rats, Environmental Toxicology 31: 1250-1263.

Lu, Z., Challis, J.K., and Wong, C.S., 2015. Quantum yields for direct photolysis of neonicotinoid insecticides in water: implications for exposure to nontarget aquatic organisms. Environmental Science Technology Letters 2: 188e192.

Lundin, O., Rundlöf, M., Smith, H.G., Fries, I., and Bommarco, R. 2015. Neonicotinoid insecticides and their impacts on bees: a systematic review of research approaches and identification of knowledge gaps. PLoS ONE 10: e0136928.

Malik, C., and Harris, R. 2014. Nonpoint source program success story: Connecticut watershed approach improves Norwalk River. USEPA Report 841-F-14-001C.

Metcalf, R.L., and Luckmann, W.H. 1994. Introduction to Insect Pest Management. John Wiley and Sons. New York, New York.

Morse, J.C. 2009. Trichoptera (Caddisflies). Pages 1015-1020 in Encyclopedia of Insects, Second Edition, V.H. Resh and R.T. Cardé, editors. Academic Press.

Mosbah, R., Djerrou, Z., and Montovani, A. 2018. Protective effect of *Nigella sativa* oil against acetamiprid induced reproductive toxicity in male rats. Drug and Chemical Toxicology 41: 206-212.

Nowell, L.H., Moran, P.W., Waite, I.R., Schmidt, T.S., Bradley, P.M., Mahler, B.J., and Van Metre, P.C. 2024. Multiple lines of evidence point to pesticides as stressors affecting invertebrate communities in small streams in five United States regions. Science of the Total Environment 815: 169634.

Noventa, S., Pace, E., Esposito, D., Libralato, G., and Manfra, L. 2024. Handling concentration data below the analytical limit in environmental mixture risk assessment: a case-study on pesticide river monitoring. Science of the Total Environment 907: 167670.

Oliver, J.B., Fare, D.C., Youssef, N., Scholl, S.S., Reding, M.E., Ranger, C.M., Moyseenko, J.J., and Halcomb, M.A. 2010. Evaluation of a single application of neonicotinoid and multi-application contact insecticides for flatheaded borer management in field grown red maple cultivars. Journal of Environmental Horticulture 28: 135-149.

Ospina, M., Wong, L.-Y., Baker, S., Bishop Serafim, A., Morales Agudelo, P., and Calafat, A.M. 2019. Exposure to neonicotinoid insecticides in the U.S. general population: data from the 2015–2016 National Health and Nutrition Examination Survey. Environmental Research 176: 108555.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team 2022. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-155.

Plafkin, J.L., Barbour, M.T., Porter, K.D., Gross, S.K., and Hughes, R.M. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. USEPA Document 444/4-89-001.

R Core Team. 2024. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rochlitz, J., Ott, D., and Scherber, C., 2024. Insect decline and its threat to migratory insectivorous animal populations. Technical Report. Convention on the Conservation of Migratory Species of Wild Animal. CMS Secretariat.

Sanchez-Bayo, F., and Wyckhuys, K. 2019. Worldwide decline of the entomofauna: a review of its drivers. Biological Conservation 232: 8-27.

Schulz, R. 2004. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: a review. Journal of Environmental Quality 33: 419-448.

Schmidt, T.S., Miller, J.L., Mahler, B.J., Van Metre, P.C., Nowell, L.H., Sandstrom, M.W., Carlisle, D.M., Moran, P.W., and Bradley, P. 2022. Ecological consequences of neonicotinoid. Science Advances 8: eabj8182.

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Chagnon, M., Downs, C., Furlan, L., Gibbons, D.W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C.H., Liess, M., Long, E., McField, M., Mineau, P., Mitchell, E.A.D., Morrissey, C.A., Noome, D.A., Pisa, L., Settele, J., Stark, J.D., Tapparo, A., Van Dyck, H., Van Praagh, J., Van der Sluijs, J.P., Whitehorn P.R., and Wiemers, M. 2014. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environmental Science and Pollution Research 22: 5-34.

Smith, R.F., and Smith, G.L. 1949. Supervised control of insects: utilizes parasites and predators and makes chemical control more efficient. California Agriculture 3: 3-12.

Spiller, N.C., Burns, K.T., Donato, M.K., and Olavarria, M. 2022. Fairfield County River Report 2022. Harbor Watch, Earthplace, Inc. 1-87 pp.

Spiller, N.C., Burns, K.T., Donato, M.K., and Olavarria, M. 2023. Fairfield County Water Quality Report 2023. Harbor Watch, Earthplace, Inc. 1-61 pp.

Stroup, W.W. 2012. Generalized Linear Mixed Models: Modern Concepts, Methods and Applications. CRC press, Boca Raton, Florida.

Suchail, S., Guez, D., and Belzunces, L.P. 2000. Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. Environmental Toxicology and Chemistry 19: 1901-1905.

USEPA 2024. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk. Accessed 21 November 2024.

USGS 2024. Estimated annual agricultural pesticide use maps. https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2009&map=IMIDACLOPRID&hilo=L. Accessed 5 December 2024.

van der Sluijs, J.P, Simon-Delso, N., Goulson, D., Maxim, L., Bonmatin, J.-M., and Belzunces, L.P. 2013. Neonicotinoids, bee disorders and the sustainability of pollinator services. Current Opinion in Environmental Sustainability 5: 293-305.

Van Deynze, B., Swinton, S.M., Hennessy, D.A., Haddad, N.M., and Ries, L. 2014. Insecticides, more than herbicides, land use, and climate, are associated with declines in butterfly species richness and abundance in the American Midwest. PLoS ONE 19: e0304319.

van Dijk, T.C., Van Staalduinen, M.A., and Van der Sluijs, J.P. 2013. Macro-invertebrate decline in surface water polluted with imidacloprid. PLoS ONE 8: e62374.

Van Lexmond, M.B., Bonmatin, H.-M., Goulson, D., and Noome, D.A. 2015. Worldwide integrated assessment on systemic pesticides. Environmental Science and Pollution Research 22: 1-4.

Walsh, C.J., Roy, A.H., Feminella, J.W., Cottingham, P.D., Groffman, P.M., and Morgan, R.P, III. 2005. The urban stream syndrome: current knowledge and the search for a cure. Urbanization and Stream Ecology 24: 585-587.

Woodcock, B.A., Isaac, N.J.B., Bullock, J.M., Roy, D.B., Garthwaite, D.G., Crowe, A., and Pywell, R.F. 2016. Impacts of neonicotinoid use on long-term population changes in wild bees in England. Nature Communications 7: 12459.