# **Neonic Pesticides in Minnesota Water:**

Their Contamination of and Threats to the State's Aquatic Ecosystems

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# **1. EXECUTIVE SUMMARY AND CONCLUSIONS**

Minnesota has seen a massive increase in the use of neonicotinoid pesticides, or "neonics," in the past few decades, driven primarily by the widespread prophylactic use of neonic coatings on crop seeds—known as "seed treatments." As a result, neonics are now frequent contaminants of water bodies across the state, likely causing significant and widespread damage to aquatic life.

Neonic seed treatments on corn and soybean seeds are the predominant neonic use in Minnesota. State agricultural neonic use likely totals more than a million pounds of active ingredient per year. Although the exact amount is difficult to ascertain because data on seed treatment use are no longer collected, it is estimated that neonic seed treatments account for 96% of all state agricultural neonic use—and 90% of total state neonic use, which includes other sectors such as landscaping and structural uses. While these nonagricultural uses are small in comparison, they enlarge the geographical reach of neonic use and, by extension, water contamination from neonics.

Prior state research and findings identified neonics as pervasive and damaging contaminants, with Berens et al. (2021) finding at least one neonic in 97% of all Minnesota creek and river water samples, and the Minnesota Department of Agriculture (MDA) identifying neonics as "surface water pesticides of concern" and stating that "seed treatments [are] the likely source of contamination in Minnesota streams and rivers near agricultural watersheds" (Petersen 2024). These findings are consistent with nationwide research linking corn and soybean production areas with widespread stream contamination from neonic chemicals used as seed treatments (Hladik et al. 2014).

This report confirms similar findings with respect to neonic water pollution in the state. Within the 12 years of MDA sampling data available, 95% of the frequently sampled (i.e., 10 times or more) flowing-water sites had at least one neonic chemical commonly used as a seed treatment, and 87% of the sites showed a mixture of two or more neonic chemicals. Where neonics were found, they appeared in most cases at concentrations expected to do biological harm. At 93% of flowing-water sites with 10 or more samples, a damage level was exceeded in at least one year of sampling. Indeed, the U.S. Environmental Protection Agency's (EPA) chronic benchmark for harms to aquatic ecosystems was exceeded in more than half of all sampled years at roughly three-quarters of frequently sampled sites. Acute impact thresholds (in the form of current European Union (EU) benchmarks—more scientifically defensible levels than the current EPA equivalents) were often exceeded also—in more than half of sampled years at 57% of the more frequently sampled sites.

MDA data indicate that neonic use across the state—particularly the use of neonic seed treatments—has resulted in nearubiquitous and chronic contamination of its water resources at levels EPA considers to be damaging. Nearly all frequently sampled creeks and rivers show contamination levels lasting for several months. Concentrations are typically elevated in the spring, reflecting the planting season, but depending on the location along the stream and distance to agricultural fields, maximum levels can occur in late summer also. In some cases, a very small amount of agricultural land can result in very high and damaging levels of contamination, even in watersheds dominated by nonagricultural land uses. This is clearly a function of the fundamental chemical characteristics of neonics—their long persistence in soils and highly mobile nature—and cannot be mitigated under current seed treatment use patterns.

While the available data and current benchmarks indicate that the expansive prophylactic use of seed treatments poses a significant threat to the health of aquatic life in Minnesota, the reality for Minnesota's aquatic ecosystems is likely much worse than shown here. There is ample evidence that current water sampling procedures fail to capture the true maximum loads to Minnesota water bodies. It is also clear that EPA's benchmark levels are inadequate; MDA itself has pointed out to EPA that assessing neonics individually (when they are usually found as mixtures) ignores their clearly additive effects and possibly even synergistic impacts (Petersen 2024). There is good evidence to show that the neonic most associated with seed treatments, clothianidin, is more toxic to aquatic life than imidacloprid, the first registered neonic. In this regard, current EPA benchmarks are in error.

Current science shows that biological impacts are real and are occurring in real time as a result of neonic water contamination. Aquatic contamination by neonic insecticides has been a worldwide problem since their introduction (Morrissey et al. 2015), and Minnesota is no exception.

A full description of issues as well as the historical context behind their registration can be found in previous detailed reports—especially Mineau and Palmer 2013 and Mineau and Kern 2023.

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# **2. NEONIC USES IN MINNESOTA**

Neonics used for crop protection include imidacloprid, clothianidin, thiamethoxam, thiacloprid, acetamiprid, and dinotefuran. Two other neonics, nitenpyram and nithiazine, are used for flea control in pets and in fly bait, respectively, and will not be considered further in this report. Most of the concern relates to the first three on this list, in part because of their toxicity and persistence but also because these have been the most widely used in North America. There are currently no registered thiacloprid products in the United States.

## **2.1 Agricultural use of the three main neonic active ingredients**

#### 2.1.1. USGS Data

Researchers from the United States Geological Survey (USGS), Thelin and Stone (2013), developed a methodology for estimating agricultural pesticide use at both state and county levels through confidential surveys of pesticide application across various crop types. These data, combined with crop acreage figures, allow extrapolation of use rates by region. Two estimates, E-Pest Low and E-Pest High, are produced. E-Pest High is particularly beneficial when pesticide use data are missing for a county, as it interpolates data from nearby counties on the basis of crop area in order to avoid unrealistic zero estimates. These interpolated results are utilized in this report.

Continuing this work, Wieben (2021) released data spanning 1992 to 2019, noting that the 2018 and 2019 figures were preliminary.

The data presented here focus on high-acreage crops (corn, soybeans, wheat, cotton, and alfalfa) and aggregated lowacreage crops (e.g., vegetables and fruit, orchards and grapes, pasture and hay, and other crops) consolidated at the state level. These estimates reflect only agricultural pesticide use, omitting domestic, landscape, and industrial applications. Not including these other uses notably underestimates the use of imidacloprid, which is extensively applied to turf and ornamental plants (see below).

After 2014, data for neonic seed treatment use were no longer collected, which is reflected in Figures 1a, b, and c. The sudden drop in in estimated use between 2014 and 2015 results from seed treatment data no longer being available, highlighting the overwhelming share of the neonic market that seed treatments account for. The use of particular neonic chemicals within particular seed markets is also clear, with clothianidin dominating the corn market, imidacloprid being popular in the soybean market, and thiamethoxam split between corn and soybeans.

**Figures 1a, b, c. USGS estimates of pounds of clothianidin (a), imidacloprid (b), and thiamethoxam (c) use by main crop group for the period 1995 to 2019. The abrupt decline in estimated use in 2015 does not reflect a drop in actual use, but rather the fact that seed treatment use data were not collected after that date.**



Imidacloprid





#### Thiamethoxam

#### 2.1.2. MDA sales data: Patterns and comparison with USGS use data

Minnesota is one of the few U.S. states where ancillary data after 2014 are available. The Minnesota Department of Agriculture (MDA) provides public access to pesticide sales and use data through its Pesticide Sales Database. This searchable database includes calculations of the total pounds of pesticide active ingredients sold in Minnesota on a yearly basis. While the data span crop chemicals from 1996 to the present and non-crop pesticides from 2006 to the present, it is important to interpret these data cautiously. Pesticides sold in Minnesota in any given year may be used in a subsequent year or may never be used within the state. More important, while Minnesota sales data should include neonic products purchased by Minnesota farmers or seed dealers to coat or "treat" crop seeds within the state, the data do not account for the use of seeds treated with neonics outside the state and imported into Minnesota. Accordingly, these data offer a poor indication of overall long-term pesticide use trends.

Comparing USGS estimates with MDA sales data yields interesting discrepancies. Because USGS usage data include seed treatments before 2015, whereas MDA sales data do not include treated seeds unless treated locally, the large discrepancy up to 2014 is easily understandable and suggests that the bulk of seeds planted by Minnesota farmers were treated outside the state.

In contrast, the 2015 to 2019 period should have yielded comparable estimates between the USGS and the MDA; but as seen in Figures 2a, b, c, and d, this is not the case. USGS use estimates are much lower than reported sales. The reason for this discrepancy is uncertain, especially since the early data (pre-2005, before the explosion in treated-seed use) appears to be much more in agreement. However, it is likely that the higher MDA pesticide sales estimates post 2015 (relative to the non-seed treatment estimates of the USGS) include the proportion of seeds treated in-state where the seed treatment product is reported to the MDA. If that is the case, current USGS data offer a more accurate estimate of the non-seed treatment use of neonics in the state.

**Figures 2a, b, c, d. Agricultural (AG) use estimates (pounds active ingredient) for Minnesota of the main neonic active ingredients and their total use. The blue lines show the USGS estimates based on grower surveys and crop area, while the red lines give the MDA reported sales data for crop use (and formulating uses). USGS estimates are valid only up to 2014. MDA reported sales appear to exclude the bulk of treated seeds in all years.** 











Both estimates post 2014 show a sharp increase in use over time, especially for imidacloprid and thiamethoxam. The MDA sales data estimate does not show much of an increase for clothianidin because the data exclude seed treatment use, and this active ingredient is almost exclusively used as a seed treatment. A comparison of pre- and post-2014 use can be used to estimate the proportion of neonics that were used as seed treatments. For example, the best fit for the USGS clothianidin data from 2004 to 2014 is an exponential increase (Figure 3). Extrapolating the fitted relationship to 2015 suggests that we should have seen a use estimate of approximately 628,000 pounds. Without seed treatments, the estimate dropped to 974 pounds of active ingredient. This suggests that 99.8% of all clothianidin use a decade ago in Minnesota agriculture was as a seed treatment.

**Figure 3. Minnesota USGS clothianidin estimated use (in pounds of active ingredient) between 2004 and 2014**  fitted to an exponential curve. The  $R^2$  value is a commonly used regression statistic that estimates how well the **fitted regression line explains the data. In this case, the curve explains 96% of the variance in the data, which is a very good fit.** 



This introduces an important question: how long was this exponential increase maintained? Taking it through to the present day, the use would extrapolate to about 7.5 million pounds of active ingredient of clothianidin, mostly on corn, but this is not realistic. Assuming that all corn is treated (based on Douglas and Tooker [2015]) and Hitaj et al. [2020]), we can do a quick reality check and see how much clothianidin corn alone could potentially account for. Existing industry websites<sup>1</sup> as well as the latest versions of pesticide labels<sup>2</sup> describe clothianidin corn treatments ranging from 0.25 to 1.25 mg/kernel. The latter is proposed for control of corn rootworm, a major pest often resulting in part from a lack of crop rotation. Based on an EPA compilation (2010), the planting rate for corn is between 26,400 and 40,250 kernels per acre. USDA statistics<sup>3</sup> suggest that a reasonable expectation for corn acreage is about 8.5 million acres for Minnesota. At the highest label and planting rate, we could therefore account for about one million pounds of active ingredient of clothianidin if it had the entire corn market share. In fact, Figures 1a, b, and c suggest that clothianidin had about 90% of the market share for corn seed treatments. Therefore, current use is not as high as the exponential increase carried through to the present time might suggest. Still, the fact that use of seed treatments does not appear to be decreasing in corn and soy, and is likely increasing in other crops such as cereals, means that the total current use of clothianidin is probably higher than it was in 2015 and well over a million pounds of active ingredient yearly.

Thiamethoxam's increase of market share appears to have been slower and is best fitted to a linear increase between 2001 and 2014 (Figure 4) with an estimated use of about 90,000 pounds of active ingredient in 2015, compared with the USGS estimate of 20,000 pounds without the seed treatments. This suggests that 82% of thiamethoxam used in agriculture was used as a seed treatment in 2014.

<sup>1</sup> E.g., Poncho® seed treatment,<https://agriculture.basf.us/crop-protection/products/seed-treatment/poncho.html>, last accessed January 7, 2024.

E.g., EPA registration no. 7969-458.

NASS QuickStats, [https://quickstats.nass.usda.gov/,](https://quickstats.nass.usda.gov/) accessed June 2024.

**Figure 4. Minnesota USGS thiamethoxam estimated use (in pounds active ingredient) between 2004 and 2014**  fitted to a linear regression. The  $R^2$  value is a commonly used regression statistic that estimates how well the fitted **regression line explains the data. In this case, the curve explains 93% of the variance in the data, which is a very good fit.**



For imidacloprid, the 2014 market appears to have peaked around 2012 and dropped slightly to around 70,000 pounds of active ingredient per year by 2015, the main seed treatment uses having devolved to clothianidin and thiamethoxam. Comparing this with the seed treatment–free estimates for the same period suggests that about 77% of imidacloprid used in agriculture was as a seed treatment.

If we look at the three main neonics and the estimated proportion of each used in seed treatments, we can conclude that in 2015, approximately 96% of all agricultural neonic use was in the form of seed treatments. More recent literature (e.g., Hitaj et al. 2020) suggests that seed treatment uses still dominate today.

## **2.2 Agricultural versus nonagricultural uses of the three main neonic active ingredients**

Table 1 summarizes the MDA sale information (in pounds active ingredient) for the three main neonic active ingredients: clothianidin, imidacloprid, and thiamethoxam.

**Table 1. MDA sales data (in pounds of active ingredient (A.I.)) for 1996 to 2022 for clothianidin (CLO), imidacloprid (IMI), and thiamethoxam (THI). Data for nonagricultural uses are not available (NA) before 2006. Finer use categories are combined into agricultural, structural, landscape, and veterinary uses.** 



a As noted in the text, some seed treatment uses may be included in the form of in-state treatment of seeds. However, the data suggest this is minimal and a small proportion of total seed-treatment use.

Even without accounting for the use of neonics as seed treatments, we can see that nonagricultural use of the three main neonics is significantly lower—typically 100 times lower—than agricultural use. Structural use of imidacloprid initially and thiamethoxam more recently is an exception, which in some years approached roughly one-fifth of the agricultural use for those chemicals. Landscape use of imidacloprid approached the range of 10% of non–seed treatment agricultural use in some earlier years. Imidacloprid is also used in veterinary products, and that use is expected to rise over time. The 2021 spike matches reports of high pet adoption rates as a result of the Covid pandemic, when as many as one in five American households adopted a pet (Bogage 2022).

Nonagricultural uses of neonics are therefore not adding much quantitatively to the overall total of neonic use in Minnesota. Seed treatment use likely accounts for more than 90% of total neonic use. However, nonagricultural neonic uses expand the geographical range of neonic contamination—especially for imidacloprid, which often is used (and detected) in urban and suburban environments.

## **2.3 Use of the "minor" neonic active ingredients**

The use of other neonicotinoid active ingredients, such as acetamiprid and dinotefuran, is relatively small compared with that of imidacloprid, clothianidin, and thiamethoxam (Table 2). Seed treatments do not significantly contribute to their usage. Thiacloprid use stopped 10 years ago in the United States and is not considered any further in this analysis.

The principal use of acetamiprid is for ornamentals, while dinotefuran is more evenly divided between structural and landscape (ornamentals and turf) uses.

**Table 2. MDA sales data for acetamiprid and dinotefuran (in pounds of active ingredient) from 2006 to 2022. Data for nonagricultural uses are not available for earlier years. Finer use categories are combined into agricultural, structural, landscape, and veterinary uses.** 



## **2.4 Increasing neonic use means increasing toxic potential for aquatic ecosystems**

Neonics have become ubiquitous contaminants of aquatic ecosystems worldwide, with demonstrated harms. A number of characteristics make them particularly problematic: they are highly persistent in soils; they are highly water soluble, migrating easily and often through runoff; they are very toxic to a broad range of species; and they are harmful at concentrations that are often too low to be detected. Their main use by volume is as a prophylactic coating applied to

crop seeds before planting (seed treatment), which research increasingly shows provides little if any economic benefit to farmers under most conditions.<sup>4</sup>

As early as 1994, EPA scientists warned that both acute and chronic aquatic risk triggers had been exceeded for both non-endangered and endangered species exposed to imidacloprid (Mineau and Palmer 2013). A full 30 years later, these predictions have come to pass and aquatic systems are being systematically degraded by neonic use in Minnesota and elsewhere. A full description of issues as well as the historical context behind their registration can be found in previous detailed reports—especially Mineau and Palmer 2013 and Mineau and Kern 2023.

While the three main neonic active ingredients are not equally toxic to aquatic ecosystems, Appendix A shows how toxicity across the three chemicals can be assessed by calculating imidacloprid-equivalent toxicity through comparative toxicity tests conducted on the same species. This approach is the preferable one given that water toxicity benchmarks are highly dependent on the extent of information available, and this varies greatly among the different molecules (see Appendix A for a full discussion). The conclusion from the analysis outlined in Appendix A is that clothianidin is the most toxic of the main three neonics—almost twice as toxic as imidacloprid. Thiamethoxam is approximately half as toxic as imidacloprid, but this estimate is made more complicated by the fact that thiamethoxam breaks down in the environment and turns into clothianidin.

Clearly, the largest potential threat to aquatic systems in Minnesota has been the exponential increase in clothianidin use, as shown in Figures 1 and 2 above. Calculating the total imidacloprid-equivalent aquatic toxicity potential of neonics used in Minnesota also makes it easier to look at the relative contribution of agricultural uses compared with other sources inventoried by MDA. For imidacloprid, the largest nonagricultural use, landscaping, contributed roughly 5,000 imidacloprid-equivalent pounds of active ingredient in recent years; structural use contributed about 12,000 imidacloprid-equivalent pounds. These amounts pale in comparison with agricultural uses (primarily seed treatments), which, as calculated above, are thought to contribute far in excess of 1,000,000 imidacloprid-equivalent pounds per year.

# **3. NEONIC WATER CONTAMINATION IN MINNESOTA**

Collecting water samples and having them analyzed for residues is the time-honored way of assessing the potential impact of pesticides on the environment. Minnesota has better monitoring data than most U.S. states. Even so, interpretation of the results of this monitoring is not straightforward. Appendix B describes the main problems with the approach; some of those considerations appear in the section below.

## **3.1 Analysis of the Minnesota water contamination data**

Data were accessed through the Water Quality Portal maintained by USGS and EPA under the National Water Monitoring Council umbrella.<sup>5</sup> This portal combines the extensive USGS database and water quality data collected by EPA for "states, tribes, watershed groups, other federal agencies, volunteer groups, and universities through the Water Quality Exchange framework."

After removal of "field blanks," control sample blinds, and replicates, a total of 25,423 distinct analyses of surface water in Minnesota were inventoried for neonics and a few of their degradates (breakdown products) between 2000 and 2024. The years 2023 and 2024 were still incomplete when the data were accessed.

<sup>4</sup> A full agronomic review is beyond the scope of this report, but see USEPA (2014), Douglas and Tooker (2015), Douglas et al. (2015), Krupke et al. (2017), and Pennsylvania State University Extension (2023) for soybean; Alford and Krupke (2017), North et al. (2017), and Li et al. (2022) for corn; MacFadyen et al. (2014) for cereal; Budge et al. (2015) and Hokkanen et al. (2017) for oilseed crops; and Clavet et al. (2014) for turf. Other reviews of the literature such as Center for Food Safety (2014, 2016), Veres et al. (2020), and Rowen et al. (2022) arrive at a similar conclusion.

<sup>5</sup> <https://www.waterqualitydata.us/>, accessed April 8, 2024.

The most comprehensive and useful sampling was carried out by the Minnesota Department of Agriculture. Only imidacloprid was looked for between 2000 and 2009. Thiamethoxam was added to the agency's sampling regime in 2010, and clothianidin in 2011. Detection limits for the three compounds, however, were high at 20–25 ng/L for most of the sampling period, a level at least twice as high as the current EPA benchmark for chronic impacts of imidacloprid and four times as high as the more scientifically based EU benchmarks of 5.7–6.8 ng/L for imidacloprid. Imidacloprid detection levels dropped to 5 ng/L in 2019; for clothianidin and thiamethoxam, they remained at 25 ng/L for the period under study in this report. In terms of looking at the full impact of the three main neonics, the years 2011 to 2022 therefore provide the best data. USGS samples were excluded from this analysis because they looked at imidacloprid only; clothianidin and thiamethoxam analyses by USGS were not reported until 2022, albeit at a much lower detection level of 0.5 ng/L. MDA, through its Monitoring and Assessment Unit in the Pesticide and Fertilizer Management Division, now reports yearly on these same data. These reports also include information on pesticides other than neonics as well as results of well water analyses outside the scope of the present report.6

#### 3.1.1. Overview of detection results

As part of its monitoring over these years, MDA reported on 165 sampling locations in various lakes and 42 sampling locations in wetlands. No neonic detections were reported for any of the lake locations and only three (imidacloprid in all cases) were detected for the wetlands. Of these 207 lake and wetland sites, 176 were represented by a single sample, 27 by two samples, and only 3 sites were sampled more than twice. While the absence of any neonic detection for any of the lakes (for which Minnesota is famous) is good news, the inference one can derive from this is limited. The real issue of concern is whether neonics will contaminate surface water if and when they are used. Without a detailed analysis of the watersheds that support these lakes and a concurrent analysis of neonic use by watershed, the significance of these nondetects is open to question.

Over the same 2000–2022 period again, MDA reported data from 279 locations described as rivers, creeks, ditches, etc. in other words, flowing water. No detections were reported for 230 of those locations. Once again however, the bulk of sites (all but 5) were sampled only once or twice. In contrast, the mean number of sampling events at sites with positive neonic detections was 66, and only 4 of the 49 sites showing positive neonic detections had fewer than three visits.

Routine water sampling as carried out in Minnesota underestimates the true maximum level of contamination as well as the probability of detection. This is discussed in more detail in Appendix B. It is clearly unreasonable to expect that grab or spot samples taken in the course of water monitoring schemes will necessarily coincide with peak concentrations of the various neonics in the monitored streams. Indeed, it has been shown that, even when taken weekly, water samples will underestimate peak concentrations by one to three orders of magnitude (Xing et al. 2013).

As discussed above, many of the sites chosen for sampling in Minnesota were sampled only once over the 12-year period examined. Even if sampling had been frequent enough to provide a true picture of expected residue concentrations (usually in the form of a distribution), there are difficulties in the interpretation of the results and clear biases when one tries to establish the proportion of samples that exceed benchmarks (Stehle et al. 2013).

Figures 4a, b, and c support the view that the ability to detect higher levels of contamination in surface waters is directly related to the intensity of sampling. Here, the maximum observed concentration of the three main neonics is plotted against the number of times the compound was detected at the site.

<sup>6</sup> See the following links for recent reports, accessed April 8, 2024: <https://wrl.mnpals.net/islandora/object/WRLrepository%3A3746>(2020 samples); <https://wrl.mnpals.net/islandora/object/WRLrepository%3A3880>(2021 samples); and<https://wrl.mnpals.net/islandora/object/WRLrepository%3A4333>(2022 samples).

**Figures 4a, b, c. Maximum value of each of the three main neonics for each sampling site plotted against the number of detections at the site. Positive relationships (at least for two of the three molecules) suggests that finding a true maximum value for a site is highly dependent on the intensity of sampling. Best fits (whether linear or exponential) are given.**



Another way of showing this relationship is simply to look at the total number of samples taken per site, regardless of whether neonics were detected, and the maximum value of any neonic detected at the site (Figure 5). The positive relationship suggests that, on average, every supplementary visit increases the maximum observed concentration of a neonic at the site by about 2 ng/L.



#### **Figure 5. Simple relationship between the number of sampling visits at any given site and the maximum concentration of any one neonic at that site.**

These analyses suggest that high neonic values detected in routine water sampling are not anomalous, but simply reflect sites with more intensive sampling. Higher sampling frequency means that high values after events such as rainfall are more likely to be detected. Following this logic, this means that most sites with neonic detections will, at some point, receive a high "slug" of insecticide capable of decimating its aquatic invertebrate fauna. We would be able to catch these moments in time given unlimited sampling effort, but this is clearly not feasible with the type of sampling in effect in Minnesota and most other jurisdictions.

In addition, water samples are often taken from large streams after much dilution has occurred, but impacts to aquatic life are expected where most of the aquatic productivity is taking place—in small drainage ditches and ponds bordering field areas to small feeder streams. Most of the MDA flowing-water sites are listed as creeks or rivers.

Of the 43 flowing-water sampling sites having a sampling frequency of 10 samples or more during the 12-year period, neonics were detected at least once at 41 of the sites; this is a 95% rate of positive detection. This projects a result much different from what would be calculated from a simple proportion of positive detection sites without regard to sampling intensity—namely 49/279, or an 18% positive detection.

It is therefore quite impossible to assess with any confidence the proportion of Minnesota creeks or rivers contaminated by neonics. Without knowing more about why specific sites were chosen, the fact that most of them did not show any residues above detection levels is not very meaningful. Sites might have been dropped after a single visit if they did not show positive detections. Even if those sampling sites were chosen based on the probability of neonic detection (because of information of nearby use or because of agricultural areas nearby), the low sampling effort undermines the value of most of the sites.

#### 3.1.2. Analysis of sites with neonic detections

To better assess the likely impact of neonic use on freshwater resources, it is more meaningful to look at sites where neonics were detected at least once in the 12 years of the current analysis. Detection indicates that there was at least some use of neonics in the watershed being sampled and/or that sampling frequency was high enough to detect residues. There is clear evidence from the literature that, where neonics are used on crops or in urban environments, they will indeed be detected in nearby bodies of water. Their long soil half-life and water solubility ensure that this is the case. As discussed above, sites with detections represent 95% of sites with 10 or more samples.

Table 3 provides a summary of the number of samples taken over the 12-year period at each of the sites with at least one positive detection, as well as the proportion of samples with levels above detection and maximum levels for each of the three main neonics. It is an almost perfect matrix in that most of the sampling visits included all three analytes. A few blank cells show that there are exceptions, with some of the samples omitting at least one of the main neonics. It is clear from the data that a mixture of neonics being seen at any given site is now the norm. Among these chosen sampling sites, clothianidin is the most prevalent contaminant, with the highest median proportion of sample detects as well as a higher level of contamination. Hladik et al. (2014) found that both clothianidin and thiamethoxam occurred more frequently than imidacloprid in Midwest streams fed from agricultural areas.

**Table 3. Summary of the number of samples taken from MDA positive sampling sites between 2011 and 2022 as well as the proportion of samples with levels above detection and maximum levels for each of the three main neonics. These sites include 41 flowing-water sites and 3 wetland sites. Thirty-nine of the sites received 10 sampling visits or more; 36 of the flowing-water sites had detections of two or all three main neonics.**



**Table 3. Summary of the number of samples taken from MDA positive sampling sites between 2011 and 2022 as well as the proportion of samples with levels above detection and maximum levels for each of the three main neonics. These sites include 41 flowing-water sites and 3 wetland sites. Thirty-nine of the sites received 10 sampling visits or more; 36 of the flowing-water sites had detections of two or all three main neonics.**



A key reason for considering only sampling sites and times when the three major products were looked for is product substitution. Over time, imidacloprid seed treatments were replaced by clothianidin and thiamethoxam. Only an assessment of the combined residues, therefore, makes sense if we are to understand current neonic contamination patterns. This point is reinforced by the data demonstrating that the vast majority of MDA's sampling sites show multiple residues. Appendix A illustrates the difficulty of having a credible impact benchmark to compare water concentrations to, especially with the less well-studied neonics such as clothianidin and thiamethoxam. For that reason, imidaclopridequivalent concentrations were calculated as described in Appendix A for each sampling site and date; the result can be compared directly with the various imidacloprid benchmarks such as the more scientifically defensible EU acute benchmark of 62 ng/L, EPA's 10 ng/L currently published chronic benchmark, or EU's 6.25 ng/L new chronic benchmark.7

Table 4 summarizes the imidacloprid-equivalent residues of the three main neonics for the main MDA sampling sites by year. The highest level of imidacloprid-equivalent residues is indicated for each year. A blank cell indicates that sampling was not carried out in that year. "BDL" means that residues, if present, were below detection limits. Given the individual detection limits, a combined concentration of the three neonics could still be as high as 75 ng/L and remain below detection levels.





7 Appendix A reviews benchmark setting in detail and discusses why EPA's methodology is not scientifically defensible, resulting in an acute benchmark that is wildly divergent from current scientific thinking. The currently published EPA acute benchmark for imidacloprid is 385 ng/L; the EU has set its acute benchmark at 62 ng/L (the average of two robust estimates: 57 ng/L and 68 ng/L). Also, it is clear that, given the demonstrated season-long contamination at sites, chronic benchmarks should be prioritized when trying to understand the damage to aquatic environments.

**Table 4. Yearly maximum imidacloprid-equivalent residues (in ng/L) for positive MDA sampling sites. A blank cell indicates that sampling was not carried out in that year. "BDL" means that residues, if present, were below detection limits.**

<b>MNDA</b> Sampling												
site	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
S002-409										13.2	25	21.3
S002-937	23.2	$\operatorname{BDL}$	$\operatorname{BDL}$	23.3	15.9	<b>BDL</b>	$\operatorname{BDL}$	<b>BDL</b>	76.1	140	6.37	BDL
S003-000								457	478	209	<b>BDL</b>	726
S003-087										<b>BDL</b>	<b>BDL</b>	198
S003-742	<b>BDL</b>	<b>BDL</b>	22.2	<b>BDL</b>	<b>BDL</b>	<b>BDL</b>	<b>BDL</b>	86.9	21.5	23	12.2	11.2
S004-032									BDL	<b>BDL</b>	<b>BDL</b>	<b>BDL</b>
S004-034			<b>BDL</b>	32.8	14.4	<b>BDL</b>	32.9	<b>BDL</b>				
S004-383			356	541	171	368	280	633	257	750	114	628
S004-839	43	<b>BDL</b>	132	132	77.5	111	102	282	266	155	187	210
S004-842	65.2	<b>BDL</b>	130	152	90.2	68.2	124	250	330	111	<b>BDL</b>	787
S004-898								<b>BDL</b>	114	217	5.02	5.25
S005-017	131	618	30.5	24	<b>BDL</b>	62.5	23.6	48.7	18.3	9.1	<b>BDL</b>	
S005-360										221	<b>BDL</b>	436
S005-376	399	72.1	836		27.4	$\operatorname{BDL}$	1018	483	27.7	276	12.9	
S005-377	69.4	<b>BDL</b>	$\operatorname{BDL}$	<b>BDL</b>						25	5.1	13.8
S005-379			84.7	<b>BDL</b>	<b>BDL</b>	167	<b>BDL</b>	190	56.2	241	<b>BDL</b>	148
S005-395		56.4	32.2	467	<b>BDL</b>	<b>BDL</b>	<b>BDL</b>	27.4	8.52	14.9		
S005-788										427	270	
S006-172										55.5	<b>BDL</b>	329
S007-314			153	250	54.9	$\operatorname{BDL}$	54.2	189	210	<b>BDL</b>	<b>BDL</b>	205
S008-847											29.6	25.9
S016-359										<b>BDL</b>		<b>BDL</b>
S016-603									148	349	135	
S016-769												216
WT00051				25.2								
WT00055				20.7								
WT00060				76.8	<b>BDL</b>							

As the sampling effort associated with the sites varies tremendously (see Table 3), the comparison of the various sites in terms of their relative level of contamination remains tentative. This is because failure to detect a high level at any one site may be a result of a lower sampling effort. As reviewed in Appendix B, true maximum levels detected in the course of monitoring exercises are always underestimated, with actual peak levels of contamination as much as 10 times higher.

Nevertheless, comparative evaluation criteria ranking the relative severity of neonic contamination per site and the likely impacts on aquatic life are proposed below (Table 5) to allow quick identification of problem sites based on the current Minnesota sampling program. As noted earlier, there were only three sites more intensively monitored by the MDA (defined as 10 samples or more during the 12-year period) where none of the three main neonics were ever detected. These sites are the only ones included in Table 5 tentatively judged to have minimal contamination.

Tentative contamination criteria were based on the median and highest of the yearly maxima recorded for the site (from Table 4) and the proportion of years in which the EU acute benchmark or EPA chronic benchmark were exceeded at the site. The less contaminated sites were those where the median or peak of yearly maxima fell below the 62 ng/L acute EU benchmark. Ratings of increasing severity were assigned to sites with median or highest yearly maxima of 1–2, 2–5 or >5 times the EU acute benchmark. The percentage of years when the EU acute benchmark or EPA chronic benchmark were exceeded were binned (in increasing degree of severity) as 0%, <50%, 50–99%, or 100%.

**Table 5. Tentative scoring of the aquatic impact potential for 47 MDA sampling sites with at least one positive neonic detection, or multiple sampling visits between 2011 and 2022 with no detections (three sites). Scoring criteria are based on yearly imidacloprid-equivalent values and exceedances of named benchmarks. Individual criteria are ranked as low (green), medium (yellow), amber (high), or red (extreme) as described in the text.** 



**Table 5. Tentative scoring of the aquatic impact potential for 47 MDA sampling sites with at least one positive neonic detection, or multiple sampling visits between 2011 and 2022 with no detections (three sites). Scoring criteria are based on yearly imidacloprid-equivalent values and exceedances of named benchmarks. Individual criteria are ranked as low (green), medium (yellow), amber (high), or red (extreme) as described in the text.** 



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One insight from this exercise is that all but three of the sites more intensively monitored by the MDA exceeded the EPA chronic benchmark in at least one of the years of sampling. In only 17% of intensively sampled sites was the scientifically defensible EU acute benchmark for aquatic life not exceeded in at least one year of sampling. In fact, the situation is likely worse because several of the sites were sampled for only one or two years. EPA's chronic benchmark was exceeded in at least one year of sampling for all but three of the intensively sampled sites in the state. This suggests neonics inflict significant and widespread damage to aquatic life in Minnesota.

Thiacloprid and acetamiprid were not detected in any sample. Dinotefuran was detected at three sampling sites. Site 005-376 (Fish Creek) in Table 5 was the only site with more than one analysis for dinotefuran. It registered multiple detections ranging from a low of 28.5 ng/L to an impressively high value of 11,700 ng/L. This adds to an already very high level of contamination from the main three neonics at the site. Two other sites had single dinotefuran detections: S005- 384 with 30.9 ng/L and S000-108 with 548 ng/L. Neither of these latter two sites showed any contamination from any of the main three neonics.

#### 3.1.3. Explaining detected contamination levels

To place the various Table 5 sampling sites in context, the sub-watershed in which each of the sampling sites is located was determined.<sup>8</sup> We then identified the proportion of each sub-watershed more likely to receive a seed treatment. This was defined as the area in field corn, oilseeds (soybean, canola, and sunflower), or cereals (wheat, oats, barley, triticale). Data from the USGS, "minimally processed" by MDA for the year 2022, were used for this.<sup>9</sup> Figure 6 shows the relationship between the median<sup>10</sup> of maximum yearly values and the proportion of the sub-watershed with a higher potential for seed treatments. It is hard not to see a relationship despite the large amount of variation and at least one notable outlier with a high level of contamination despite almost no area in the aforementioned crops. The wide degree of variation was expected. This crude analysis does not take into consideration where the bulk of the high seed treatment crops are located in the sub-watershed relative to the water sampling station. This would require a much finer analysis.

I am indebted to Maeve Sneddon at NRDC for the GIS analysis of watersheds and land use.

<sup>9</sup> <https://gisdata.mn.gov/dataset/agri-cropland-data-layer-2022>, accessed 21 May 2024.

<sup>10</sup> I chose the median because it is a more "stable" measurement than the maximum value—i.e., less likely to be affected by a single anomalous year.

The outlier is MDA site S005-376, located on Fish Creek, a tributary to the Mississippi and downstream from a 62 acre peri-urban park. The site was mentioned earlier as it recorded the highest peak neonic value recorded in the state (1,920 ug/L of thiamethoxam in August 2017), the highest median yearly maximum of all MDA sites, as well as very high dinotefuran levels. Clearly, a very small amount of agricultural area (99.5% of the sub-watershed area is designated as "non-crop") can result in a very high level of contamination, although the overall statewide trend is as expected. However, areas of field crops with a high potential for seed treatment use tend to show higher neonic contamination levels. This confirms that the use of seed treatments is a major risk factor for aquatic impacts in Minnesota.

**Figure 6. Simple plot of the median value of yearly maxima of imidacloprid equivalents (ng/L) for water sampling sites detailed in Table 5 against the proportion of land area most susceptible to receive seed treatments as defined in the text.** 



#### 3.1.4. Residues over time at a selected sampling site—Site S004-383, Zumbro River

The location of MDA sampling point S004-383 is the North Fork of the Zumbro River upstream from the town of Wanamingo at County Road 30 (Figure 7). This is one of the more intensively sampled of all MDA sites and offers the best opportunity to examine the temporal extent of contamination in any one year. This is an interesting site also because examination of a satellite map shows that much of the upstream stretch of the river is flanked by a treed buffer. In theory, this should mean that contamination from nearby fields is reduced. The site is partly fed from Spring Creek, originating from the 65-acre North Fork Zumbro Woods Scientific and Natural Area, and one would assume that this area receives minimal neonic contamination.

**Figure 7. Satellite map of MDA sampling point S004-383 (courtesy of Google Earth).**



Figure 8 shows the contamination levels over 10 years of sampling at the site. These levels are simple summed residues of all neonics without correction for the toxicity of the individual molecules as in sections above.





What this plot reveals are repeated contamination events of the stream by a mixture of neonics. Drilling down into the information for the most intensive years of sampling—2019, 2020, and 2022 (13, 11, and 12 sampling visits, respectively)—adds to our understanding of the temporal extent of the contamination (Figures 9a, b, c). Note that zero values are used here for values below detection limits; however, the actual detection limit for combined residues could be as high as 75 ng/L in most years, as discussed earlier. The data indicate that contamination in the river is present all summer long, reinforcing the view that a chronic benchmark is the proper one to use to assess damage to aquatic ecosystems. Peaks can be in the mid-May to early-June period corresponding to seeding but can also be recorded as late as August. The cumulative toxicity potential of neonics—i.e., the ability of many small neonic exposures to add up, causing greater and greater harm over time—has been well studied. In a recent expansion of previous analyses, Sánchez-Bayo and Tennekes (2020) demonstrated quite convincingly that neonics show irreversible cumulative toxicity in both aquatic and terrestrial invertebrates. Their sound analysis argues for the fact that any benchmark based on acute or even short-term toxicity data is completely irrelevant in a real-world exposure situation and that peak values are the best indication of biological damage.







 $08 - 17$ 

 $07 - 28$ 

 $07 - 08$ 

09-06

## **3.2 Independent analyses of neonic water contamination in Minnesota**

#### 3.2.1. Berens et al. 2021

Berens et al. (2021) provided a snapshot of neonic water contamination in the state for 2019. Sample collection was said to be coordinated with ongoing monitoring programs where possible; river and stream sampling specifically was said to be coordinated with the MDA. One key difference with the results highlighted above was that all three main neonics were detected in lake samples. Those lakes were in moderately to highly urbanized parts of a single county and chosen on the basis of a history of green algal blooms.

Berens et al. reported that 97% of all creek and river samples contained at least one neonic, with clothianidin being the most frequently detected at either agricultural or urban sites but with higher median concentrations at agricultural sites. Imidacloprid levels, however, were higher at urban sites—as would be expected from its use pattern. Acetamiprid was detected at one river site (Sauk River—Site S000-017 above); this is explained by lower detection levels than in the MDA analyses reported here.

Berens et al. pointed out that lakes are subject to the same considerations as rivers in terms of runoff. They saw a signature in lakes similar to what they observed in creeks and rivers from early-season seeding. Concentrations were generally lower in the sampled lakes; it is likely that their agricultural drainage area was smaller than it is for creeks and rivers.

Berens et al. also reported on neonic concentrations (mostly imidacloprid) from wastewater treatment plants (WWTPs), showing that these chemicals are not removed, or are only partially removed, by treatment. Indeed, Xie et al. (2021), working at California WWTPs, estimated that 92% of the imidacloprid entering the WWTP went through untouched. Imidacloprid concentrations alone (before other neonics are added to the mix) were above the EPA chronic benchmark at all sampled WWTPs based on single 24- to 48-hour composite samples taken in late June.

#### 3.2.2. Petersen 2024 (MDA)

Most recently, the MDA summarized its own data on neonics in response to EPA's request for comments on requirements applicable to treated seeds (Petersen 2024). This letter states that clothianidin and imidacloprid were identified as "surface water pesticides of concern" in 2020 as a result of monitoring results. (Thiamethoxam was not included because it did not exceed EPA's benchmarks for the protection of aquatic life. However, Appendix A of this report shows the flaws in current benchmark determinations.) The letter identifies a relationship between reported bee kills and surface water detections with seed treatment use at planting time. Benchmark exceedances are significantly associated with the planting and early growing season, "*implicat[ing] seed treatments as the likely source of contamination in Minnesota streams and rivers near agricultural watersheds.*" The letter also points out that EPA underestimates the risk to aquatic systems by looking at neonics individually when they are usually found as mixtures. Finally, the MDA completed its analysis by calculating 21-day average concentrations of clothianidin and imidacloprid and showing that these concentrations frequently exceed the chronic EPA benchmarks. This is despite the fact that the EPA benchmarks were taken at face value; for clothianidin, this was set an order of magnitude too high based on the available science reviewed in Appendix A. Also, as we observed earlier, the cumulative toxicity of neonics argues for looking at peak values rather than long-term averages in concentration.

# **4. ACKNOWLEDGMENTS**

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## **A.1. EPA's water quality benchmarks: Background and shortcomings of EPA's methodology**

To assess a chemical's potential impact on aquatic systems, it is essential to estimate the concentration in water at which adverse effects on aquatic life are expected, referred to as a "benchmark" value. This value is typically set by gathering toxicity test data—ideally from a diverse range of organisms—and extrapolating from these data to derive a single metric that protects the aquatic ecosystem as a whole. Different jurisdictions often derive different benchmark values for the same chemicals due to varying approaches.

EPA (U.S. Environmental Protection Agency) has traditionally used a single test value for what it terms the "most sensitive" species, i.e., the lowest acute or chronic toxicity value among those available. This approach implies comprehensive protection for all species but can be misleading due to its dependence on often limited testing. Even closely related species can exhibit significant differences in sensitivity to pesticides or other chemicals. The likelihood of identifying the "most sensitive" species is much higher if many species are tested rather than just a few. However, datasets for newer pesticides are typically too small (sometimes comprising only one or two species) to reliably identify the true "most sensitive" species in ecosystems that contain thousands. Consequently, even where contamination levels are maintained below such a benchmark, aquatic systems can, and often do, suffer damage.

Recognizing these issues, most other jurisdictions or regulatory bodies have adopted alternative strategies. One approach involves placing all available toxicity endpoints (e.g.,  $LC_{50}$  values—the concentration expected to kill half of the tested organisms) on a mathematical distribution and selecting a single value based on the proportion of values expected to fall below this chosen point. The 5% tail of a distribution is often used as the benchmark, although sometimes the 10% or 15% tail is selected. In addition, this tail value can be estimated with a high (e.g., 95%) or low (e.g., 50%) probability of not being overestimated and leaving several species without the needed protection. Methods have been developed to approximate the results of a distribution analysis when there are too few values to plot a distribution. An alternate strategy is to acknowledge that the "most sensitive" species cannot logically be determined and that even distribution analyses have limitations, particularly with small sample sizes. Thus, an extrapolation or safety factor (either arbitrarily derived or more frequently based on experience with similar datasets) is applied to the lowest value found in the tested species sample or to a value derived by curve-fitting as described above. Using a safety factor also accounts for the possibility that wild organisms may be more sensitive than laboratory test organisms for various reasons. Of all these approaches, EPA's is the least protective and the least scientifically defensible.

A comprehensive examination of the process for setting reference levels for imidacloprid and other neonics was detailed in a series of reports focusing on New York State (Mineau 2019) and California (Mineau 2020). These reports argued that EPA has systematically underestimated the toxicity of clothianidin and thiamethoxam (and other, lesser-known neonics), as it had initially done with imidacloprid. A significant finding was that, as of 2017, with 36 aquatic invertebrate species tested, sensitivity to imidacloprid varied by a factor of 790,000 from the least to the most sensitive aquatic insect or crustacean. Thus, setting any benchmark based on a "most sensitive" species from smaller datasets on other neonics is as scientifically rigorous as a roll of the dice, even after EPA applies a factor of  $2$  to the lowest recorded test value.<sup>11</sup> EPA does use species sensitivity distributions in some cases, although its approach is not consistent. This has led to inconsistencies in the benchmarks and misguided views as to which neonic is the most toxic to aquatic life. In contrast, the EU has a much more scientific approach to deriving aquatic toxicity benchmarks (European Commission 2018). Its approach uses species sensitivity distributions where possible, although assessment factors are still used on the results to reflect the quantity and quality of available data.

<sup>11</sup> [https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk.](https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk)

As an example, Tables A.1 and A.2 summarize the long and checkered history of acute and chronic freshwater benchmarks for imidacloprid derived by regulatory agencies and scientists on the basis of data available to them at the time. This is to illustrate the difficult and arbitrary nature of setting protective benchmarks, even for a pesticide as intensively studied as imidacloprid.



**Table A.1. A historical summary of acute imidacloprid benchmarks for freshwater invertebrates.**

12 Stands for Hazardous Concentration at the 5% tail of a distribution of concentration values—here for a distribution of LD50 values.







**Table A.2. A historical summary of chronic imidacloprid benchmarks for freshwater invertebrates.**

It is clear that, as time progressed, EU regulators grew increasingly concerned about the aquatic impacts of imidacloprid contamination—but not so their North American counterparts. Most chronic benchmarks developed in the EU have hovered around 10 ng/L or lower for a number of years now; it has most recently been set as low as 2.4 ng/L under the European Union's water framework initiative. The published EPA 10 ng/L benchmark is reasonable in this context. It is in line with current thinking by many experts worldwide and appears to fit the current field evidence. In their regulatory function, however, both EPA and the Canadian PMRA have become less stringent and are now recommending higher benchmarks and reduced protection for aquatic systems.

It is sobering to realize the significance of a 2.4 ng/L benchmark—the one developed by SCHEER in 2021—when most of the analyses presented in this report had detection limits of 25 ng/L, 10 times higher.

#### A.1.1. EPA's neonic aquatic life benchmarks are currently far less protective than those established in the EU.

Aside from perhaps the currently listed imidacloprid chronic benchmark of 10 ng/L, EPA benchmarks for the main neonic insecticides are still out of step with those of European regulatory agencies and, as argued in the text on methodological grounds, not sufficiently protective.

As table A.3. below demonstrates, the benchmarks developed by EU regulators are all more protective than those used by EPA, often by more than an order of magnitude for the main three neonics at least. The case of the Canadian PMRA is a bit stranger. The latter, following an extensive review of aquatic toxicity of the three main neonics between 2016 and 2018, had benchmarks more closely aligned with those of European regulators. However, following the publication of their proposed decision to cancel many registrations because of aquatic concerns, and after consideration of industry comments, chronic benchmarks were radically increased—by approximately 10-fold in the case of clothianidin and thiamethoxam. The PMRA no longer proposes to cancel any of the three main neonics.



a Data obtained from:<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk>(Consulted November 2024; but said to have been updated 22 October 2024). However, these do not reflect more recent assessments such as the USEPA 2022 endangered species assessments (see text).

b Available online from: [https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/public/protecting-your-health-environ](https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/public/protecting-your-health-environment/programs-initiatives/water-monitoring-pesticides/aquatic-life-reference-values.html)[ment/programs-initiatives/water-monitoring-pesticides/aquatic-life-reference-values.html](https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/public/protecting-your-health-environment/programs-initiatives/water-monitoring-pesticides/aquatic-life-reference-values.html) (Consulted November 2024; but said to have been updated 15 May 2024).

c Based on PMRA (2021); revised from 360 ng/L in PMRA (2016).

d Based on PMRA (2021); revised from 41 ng/L in PMRA (2016).

e Revised from 26 ng/L (based on a distributional analysis) in PMRA (2018a).

f Revised from 1.5 ng/L (based on a distributional analysis) in PMRA (2018b).

g The range in values reflects the use of different methodologies—deterministic versus probabilistic.

- h SCHEER 2021.
- i SCHEER 2023a.
- SCHEER 2023b.
- k SCHEER 2023c.
- l SCHEER 2023d.

m EU (European Union) 2014. Dinotefuran is registered as a biocide in the EU. Although not a formal benchmark, the European Union has set this PNEC (Predicted No Effect Concentration) for risk assessment purposes.

None of the benchmarks for any of the agencies, however, address the issue of multiple neonic residues at sampling sites, which is commonplace in the United States as it is in many other jurisdictions. To assess the real risk of aquatic impacts and to avoid issues regarding smaller datasets (applicable to all the neonics but imidacloprid), I believe a comparative approach is more fruitful (as discussed below).

#### A.1.2. EPA unjustifiably discarded its own imidacloprid aquatic life benchmarks in its recent endangered species assessments

Recent developments in the U.S. risk assessment world merit a short discussion. By 2016, EPA had finally adopted benchmarks for imidacloprid that were more in line with those of other regulatory bodies in Europe and Canada—namely 10 ng/L. However, under the guise of standardizing and improving data quality, recent assessments by EPA on the toxicity of neonics to threatened and endangered aquatic life unjustifiably used less protective risk assessment benchmarks

(USEPA 2022).13 This shift abandoned the measurable harms or "endpoints" that EPA previously relied on to assess pesticide threat to aquatic species, and also intentionally excluded a number of studies identifying the harms of neonics to species at exceptionally low concentrations.

In its previous assessment of imidacloprid, EPA (USEPA 2016) supported the use of "immobilization"—i.e., the pesticide concentration at which organisms were paralyzed and rendered nonfunctional from an ecological standpoint—as the appropriate endpoint, stating that "*the effects of imidacloprid (and other neonicotinoids) on mayfly immobilization occur at substantially lower levels than lethality. Specifically, LC50* [lethality] *values ranged from 6.7 to 154 µg ai/L for* C. dipterum *and*  C. horaria whereas  $EC_{50}$  [immobilization] values varied from 0.77 to 32 µg ai/L for these same species."

It also stated that "*immobilization is considered an ecologically relevant apical endpoint for characterizing the acute effects of pesticides, especially neurotoxic insecticides, on aquatic organisms.*" (USEPA 2016, p. 74)

This assessment method is consistent with that of most aquatic toxicologists. Yet, in its most recent assessment of the three principal neonics concerning threatened and endangered species—i.e., those most vulnerable to extinction— EPA inexplicably adopted new data exclusion principles and altered how study endpoints are evaluated to make its assessments less protective. Despite previously emphasizing the ecological importance of immobilization in laboratory tests, the agency favored mortality endpoints over immobilization. It also imposed stricter conditions on studies, leading to the exclusion of many independent university research studies: "*If a definitive immobility and mortality endpoint was available from the same test, the mortality endpoint was used (because immobility is intended as a surrogate for mortality)."* 

In addition, stricter "quality" criteria were used, such as a *"minimum of four concentrations of technical grade active ingredient, plus appropriate controls, tested within each study.*" (USEPA 2022, Appendix 2-5, p. 3)

In this revised assessment, EPA did not reference any of its previous assessments or explain the rationale behind rejecting immobilization as a critical endpoint or dismissing test data based on formulated material as opposed to technical-grade material. This shift means that industry tests now hold more weight in toxicity assessments, as independent researchers often lack access to technical-grade material.

While EPA (USEPA 2022) claims that its data selection process introduces more scientific rigor in deriving benchmarks, it also commits a serious methodological error by including multiple data points for the same species, thereby skewing the distribution. For example, the cladoceran species *Daphnia magna*, which is known to be highly insensitive to neonics, is included six separate times in the distribution analysis (USEPA 2022, Appendix 2-5).

For its 2016 acute toxicity standard for imidacloprid (USEPA 2016), EPA had utilized immobilization values from three ephemeroptera species, ranging from 650 to 1,400 ng/L. The 650 ng/L value (from Alexander et al. 2007) was deemed "qualitative" due to the lack of raw data, while the 770 ng/L value from Roessink et al. 2013 was adopted as the freshwater acute standard. These tests were conducted with formulated materials (typical end-use products, or TEP). The acute toxicity benchmark of 385 ng/L was derived by applying a safety factor of 2 to this "quantitatively acceptable" mayfly endpoint, acknowledging the likelihood of more sensitive, yet untested, species. In the same assessment (USEPA 2016), a species sensitivity distribution of 32 acute values produced an  $HC_s^{14}$  of 360 ng/L. The close agreement between these two values likely reassured EPA scientists, who then used the 385 ng/L acute benchmark, which still appears on the agency's website. Additionally, in the same report (USEPA 2016), EPA dismissed an attempt by Bayer Crop Science (cited as Moore et al. 2016) to establish an  $\rm{HC_s}$  value at 1,730,000 ng/L, citing clear bias in the selection of acceptable data points.

Yet, without justification, EPA adopted a significantly less protective acute imidacloprid benchmark in line with the industry proposal rejected earlier—1,430 ng/L (1,100 ng/L for insect species) and 13,150 ng/L for freshwater and saltwater invertebrate species, respectively—for its assessment of mortality to threatened and endangered aquatic or consumer species. This change was based on  ${\rm HD}_5$  values after a distribution analysis of carefully selected data, substituting mortality for immobilization where possible, and excluding tests with formulated material or insufficient dose levels. A sublethal maximum acceptable toxicant concentration (MATC) of 280 ng/L based on the most acceptable chronic study is also used in the risk calculations (USEPA 2022, Appendix 4-2). This revisionism contrasts starkly

<sup>13</sup> Risk assessment endpoints and benchmarks established by EPA for harm to aquatic life clearly serve two different purposes. However, one may question the logic of using risk assessment endpoints that are radically less protective than established benchmarks of protection, especially when the assessment is for an endangered species.

<sup>14</sup> Hazardous Concentration (in this case LC50 value) at the 5% tail of the fitted distribution, as explained earlier.

with the European Food Safety Authority's assessment of imidacloprid, which revised its acute benchmark downward, pegging it at 57 or 65 ng/L depending on the method followed. If this new EPA interpretation were to stand and replace the current EPA published benchmark, this would represent a more than fourfold difference in what is considered a safe concentration in Europe versus the United States, based on the prevention of sublethal impacts in the case of endangered species, or more than a twentyfold difference for lethal effects on individuals of unlisted species.

While EPA appears to continue to endorse its 2016 385 ng/L benchmark on its website, the benchmark is effectively meaningless if it is discarded any time actual protection or mitigation is required. It is ironic that this reversal and effective "downgrading" of the toxicity of imidacloprid occurs in the context of an assessment intended to protect endangered species.

## **A.2. Toward more defensible aquatic toxicity benchmarks: How do neonics compare in their aquatic toxicity?**

In our prior report (Mineau and Palmer, 2013), we advocated that the aquatic toxicity of thiamethoxam and clothianidin to aquatic insects and crustacea should be regarded as akin to that of imidacloprid, based on comparisons of toxicity tests conducted on the same species with different neonics. This assertion was reaffirmed and bolstered by Morrissey et al. (2015), who concluded: "*In general, acute and chronic toxicity of the neonicotinoids varies greatly among aquatic arthropods. . . . Based on limited data, however, it appears that differences in relative toxicity among the various individual neonicotinoids are minor.*" (Morrissey et al., 2015)

Other scholars have also remarked on the comparable toxicity of imidacloprid and second-generation neonics like clothianidin and thiamethoxam, e.g., Hoyle and Code (2016), leveraging newer data such as that of Cavallaro et al. (2017—but accepted for publication and data made available in 2016). The latter obtained comparative data for the three neonics on the same chironomid species, revealing nearly identical toxicities for imidacloprid and clothianidin, albeit slightly less for thiamethoxam.

Publication of additional comparative data by Raby et al. (2018a, 2018b) finally furnished enough information to convince EPA that differences among neonic active ingredients were indeed minimal (USEPA, 2020a):

 "*When considering the toxicity data for the mayfly, all four chemicals are similar, with clothianidin, dinotefuran, and thiamethoxam all having 95% confidence intervals that overlap with the confidence intervals of imidacloprid. For the midge, there are slight differences in toxicity among the chemicals, where both clothianidin and imidacloprid are similar (95% confidence bounds overlap), and dinotefuran and thiamethoxam are slightly less toxic (LC50 values are 2x and 5x higher than imidacloprid; confidence bounds do not overlap with those of imidacloprid or clothianidin).*" (USEPA, 2020a)

Similar findings emerged from chronic toxicity tests, with thiamethoxam being marginally less toxic than imidacloprid, albeit by only a twofold difference. This is reflected in current EU benchmarks (see table A.3). It is noteworthy that thiamethoxam breaks down into clothianidin, thus diminishing the ecological relevance of its lesser toxicity. No-effect concentrations15 for clothianidin and imidacloprid were within a factor of 4 and 2 for the most sensitive and second-most sensitive species, respectively. Clothianidin proved more toxic than imidacloprid to the most sensitive species (a mayfly) but less toxic than imidacloprid for the second-most sensitive species, a chironomid. Maloney (2018b) found that under simulated field conditions, chironomid populations were equally affected by imidacloprid and clothianidin, while thiamethoxam appeared to be about one-tenth as toxic.

Evidently, the differential toxicity attributed to the three main neonic active ingredients in past and present EPA aquatic risk assessments lacks scientific justification. Indeed, EPA has contradictory views on the relative toxicity of neonics. While recognizing that, when fairly compared, their toxicity to aquatic life is similar (at least for the three main compounds), the official aquatic benchmarks are still very far apart. **For example, the clothianidin acute benchmark is 45-fold less protective than that of imidacloprid when, in fact, clothianidin is nearly twice as toxic when tested on the same assemblage of organisms.** Whether imidacloprid or the second-generation clothianidin demonstrate greater

<sup>15</sup> This is the level in a toxicology study where the endpoint being sought, e.g. lethality, is not seen. The no-effect level is highly dependent on the sample size used in the test as well as on the specific test conditions. It is less reliable than a computed LC50, for example.

across-the-board toxicity also depends on whether acute or chronic values are being considered (see Table A.4), further underscoring the inadequacy of the EPA methodology and the agency's disparate benchmarks for the chemicals.

At a minimum, neonics should be deemed of equivalent toxicity until proved otherwise (but see analysis below). This includes dinotefuran, the neonic active ingredient for which we have the least data. Given that water samples typically contain several neonic residues, an additive model of effect serves as a pragmatic starting point for evaluating the genuine impacts of neonics. However, we can propose better than a straightforward addition of residues with the three principal neonics for which more aquatic data have accumulated. I believe there are now sufficient data to work out toxicity equivalency factors for imidacloprid, clothianidin, and thiamethoxam.

The EU recently gathered available aquatic toxicity on the three main neonics and calculated distribution-based endpoints (e.g.,  $\rm{HC}_{_S}$  values calculated with a high confidence that they have not been overestimated) for both acute and chronic tests (SCHEER 2021, 2023a, 2023b). These are the same datasets that formed the basis of the new EU benchmarks (Table A.4).



a Note that this value was not retained by EU authorities because of poor distributional fit and wide confidence limits.

I used those vetted compilations of toxicity tests assembled for all crustacean and aquatic insect tests (SCHEER 2021, 2023a, b) to fairly compare the toxicity of the main three neonics to the same species. Most of the comparative tests were conducted in the same laboratory and therefore provide the best information on relative toxicity.

Acute toxicity tests were used for this analysis, both because there are more available comparisons and because chronic test conditions are more likely to diverge over time. Data were matched for test conditions, and only studies with the highest reliability ratings assigned by the EU were used. Test results were standardized by assigning a value of 1 to imidacloprid results.  $EC_{50}$  (immobilization) or  $LC_{50}$  results are compared separately because these are often generated from the same studies and would not be independent. The vast majority of the comparisons are from the same laboratory, most from Raby et al. (2018a, 2018b), mentioned earlier.

#### **Table A.5. Comparison of acute crustacean and insect tests on the main neonics. Statistics derived for relative LC50 values, imidacloprid being set as 1.**



I would argue that the medians of the  $EC_{50}$  ratios provide the best starting point for establishing toxicity equivalents when adding up residues in any one sample. EC<sub>50</sub> refers to paralysis or immobility of the test organism; this is easier to measure than mortality in some organisms. Also, it is the ecologically relevant measure in terms of ensuring a functioning aquatic ecosystem as argued by EPA in 2016 (but not in its 2022 assessment for endangered species), especially in a river system where affected individuals will be swept downstream if paralyzed (invertebrate drift). As the mean values are clearly influenced by a few extreme values, when the mean ratio is considered, clothianidin jumps from being nearly twice as toxic as imidacloprid to being a little under half as toxic. I posit that those same extreme values are responsible for the different probabilistic-based analyses, and that the median value provides the best insight as to the true relative ecological toxicity of these two chemicals. At the end of the day, a higher proportion of the tested species (69% based on  $EC_{50}$  values) are more sensitive to clothianidin than to imidacloprid.

On that basis, toxicity equivalency factors of 1.9 for clothianidin (reciprocal of 0.52) and 0.53 for thiamethoxam (reciprocal of 1.9) are indicated. This means that clothianidin is roughly twice as toxic as imidacloprid, while thiamethoxam is roughly half as toxic. This differential is also consistent with the spread between their relative chronic toxicities. Again, the wide differential in current EPA benchmarks is not warranted and is indicative of poor methodology compounded by unequal datasets.

It is important to note that thiamethoxam is a proto-neonic and that much of its insecticidal activity comes from the fact that, after it is applied, thiamethoxam metabolizes to clothianidin in the environment. In terrestrial environments, the yield of clothianidin from thiamethoxam is about 66% (European Commission 2006). It is not clear from the literature what the conversion of thiamethoxam to clothianidin in the external and internal environments of exposed aquatic invertebrates is likely to be. Therefore, the factors proposed here will be used to provide toxicity values in imidacloprid equivalents, recognizing that the impact of a mixture containing thiamethoxam is likely greater than calculated because it readily converts to clothianidin in the real world.



**Table A.6. Comparison of individual species tests for the three main neonics. Toxicities in ug/L. Values with a high degree of reliability (1 or 2 in the EU scheme) were retained. When there were repeat measurements for the same endpoints under the matching conditions, a geometric mean of the values was computed.**



**Table A.6. Comparison of individual species tests for the three main neonics. Toxicities in ug/L. Values with a high degree of reliability (1 or 2 in the EU scheme) were retained. When there were repeat measurements for the same endpoints under the matching conditions, a geometric mean of the values was computed.**



#### A.2.1 Possible future refinements in assessing the comparative toxicity of neonics

The exercise above to place the neonics on an equal "standing" considers only the relative toxicity of the different compounds. In the real world, however, the likely aquatic impacts will depend also on the ease with which residues enter the aquatic environment. The potential for pesticides to be found in surface runoff depends on their water solubility, ability to bind to soil, and persistence in soils. Pesticide industry scientists (Chen et al. 2002) developed a validated indicator of runoff potential called the Surface Water Mobility Index, or SWMI. This index ranges from 0 (for low mobility) to 1 (for high mobility). These index values are calculated in Table A.7 based on properties obtained from the Pesticide Properties Database. On that basis, at least three neonics, including the two main seed treatment chemicals (clothianidin and thiamethoxam), are expected to be more likely to run off to surface water than imidacloprid. On that basis, the higher toxicity of clothianidin would be further exacerbated and the lesser toxicity of thiamethoxam would not be as advantageous as suggested, given that it is the most mobile of the three.



**Table. A.7. Surface Water Mobility Indices (SWMIs) for neonicotinoid insecticides based on an algorithm designed by Chen et al. (2002).a**

a Input data from Pesticide Properties Database at [https://sitem.herts.ac.uk/.](https://sitem.herts.ac.uk/)

### **A.3. Additivity or synergisms**

Monitoring data makes it clear that a compound-by-compound approach, as currently employed by American and Canadian regulatory bodies, is not tenable in light of the frequent detection of multiple residues across various aquatic ecosystems. Morrissey et al. (2015) similarly advocated for assessing summed residues, contending that toxicity benchmarks were proximate enough to warrant a joint toxicity benchmark.

Contradictory findings emerged from studies by Maloney et al. (2017, 2018a, 2018b) regarding compound additivity. While laboratory experiments on a chironomid species seemingly demonstrated a greater-than-additive effect with combinations of imidacloprid, clothianidin, and thiamethoxam, outdoor experiments in pond mesocosms yielded no evidence of synergistic effects among compounds. Nevertheless, impacts on chironomid emergence generally exceeded predictions from laboratory data, albeit with considerable variability among pond replicates, rendering interpretation challenging. Intriguingly, Bayer Corp., a major neonicotinoid manufacturer, had suggested potential synergistic action among several neonicotinoid insecticides, obtaining a patent on this discovery (Bayer Crop Science 2010).

In a seminal study published in *Science*, Schmidt et al. (2022) merged field observations from 85 coastal California streams with mesocosm testing of the dominant neonics, imidacloprid and clothianidin. The abundance of mayflies (all species combined) was evidently impacted by both compounds, with a 50% reduction observed at time-weighted average concentrations (over 30 days) of 1,050 ng/L and 1,350 ng/L for imidacloprid and clothianidin, respectively. Notably, examination of cumulative emergence over time suggested discernible effects at concentrations as low as 1 ng/L for clothianidin, thiamethoxam, and, to a lesser extent, imidacloprid (Figure 2 in Schmidt et al., 2022), representing levels significantly lower than EPA's current chronic benchmark for imidacloprid reviewed above.

Integrating their findings with existing chronic studies, Schmidt et al. (2022) derived chronic HC<sub>5</sub> values of 17 ng/L for imidacloprid and 10 ng/L for clothianidin but suggested that these values may not adequately preserve cumulative mayfly emergence, thus warranting a reassessment of neonic toxicity, as discussed earlier regarding time-weighted toxicity.

Through their experimental streams (mesocosms), the authors confirmed that imidacloprid and clothianidin exhibited greater-than-additive behavior, acting synergistically in many instances. Field samples revealed that total mayfly extirpation occurred at concentrations of imidacloprid or clothianidin that caused only a 50% decline in abundance with either compound alone in mesocosm settings.

Neonic mixtures were detected in 56% of streams, with at least one neonic detected in 72% of sampled streams (N=85). Summed neonic residues reached concentrations as high as 5,760 ng/L. Imidacloprid often dominated the mixture, yet dinotefuran was the most frequently detected, and thiamethoxam registered the highest concentration. The authors noted that at least one of the EPA benchmarks (see online levels in Table A.3) was exceeded in 28% of the samples. All samples were collected during April–June 2017 under low-flow conditions, potentially missing peak residue levels following rainfall, although they did cover the period when larval communities are well developed.

In the previous reviews and analyses referenced above (Mineau and Palmer 2013, Morrissey et al. 2015, Mineau 2019, 2020) we argued that, because of their persistence (demonstration of season-long presence in monitored bodies of water) and near-cumulative effects shown in invertebrate tests, the chronic benchmark is the ecologically relevant one to use when assessing risk from monitored water concentrations. We stand by that assessment.

## **A.4. Structural issues persist in EPA's assessment of neonicotinoids in aquatic systems**

In addition to the significant issues previously discussed, there remain fundamental problems with how EPA is assessing neonicotinoids in aquatic environments. These core issues have been highlighted repeatedly but have yet to be addressed or even acknowledged by EPA or other regulatory agencies.

The most critical issue is the ongoing failure to consider the time-dependent nature of neonic toxicity. Tennekes (2010) was the first to propose that neonics act as "one-hit" chemicals, exhibiting nearly perfect cumulative toxicity. This implies that a small dose can be as hazardous as a larger one if the exposure duration is extended. This concept has been reiterated multiple times, most recently by Sánchez-Bayo and Tennekes (2020). Neonic residues have been detected in watersheds for more than a year post-application. Consequently, even chronic toxicity benchmarks, which are based on 21- to 28-day tests, are inadequate. Following this logic, impacts on aquatic life are expected at levels far below the established chronic toxicity thresholds. Furthermore, experimental evidence suggests that even brief pulses of neonics can result in delayed mortality in exposed aquatic invertebrates, an effect not captured by current testing protocols.

Both of these issues pose a significant challenge to the current assessment methods for neonics. However, to my knowledge, EPA and other regulatory bodies continue to disregard these findings. Despite having more than a decade to address these concerns, no action has been taken. The question remains: why is this not happening?

Additionally, EPA continues to evaluate the toxicity of neonics to freshwater and saltwater organisms separately. Our 2013 report argued that the available science does not support this distinction. The perceived lower sensitivity of saltwater or brackish species is likely due to a lack of toxicity data. This oversight potentially places species-rich estuaries and other coastal areas at a much higher risk than currently acknowledged. In its recent assessments, the EU has placed much more stringent benchmarks on saltwater environments because of the paucity of data (e.g., SCHEER 2021 for imidacloprid). A safety factor of 10 was agreed on after the data for freshwater and saltwater organisms were combined. In contrast to the way North American regulatory bodies carry out aquatic protection, the EU applies the 'Precautionary Principle' when data are lacking.

## **A.5. How is the aquatic risk of neonics currently viewed in the wider scientific community?**

A notable analysis that closely followed our earlier report (Mineau and Callaghan 2013) was the Worldwide Integrated Assessment of the Impact of Systemic Pesticides on Biodiversity and Ecosystems (WIA). This assessment, conducted by an international group of scientists, reviewed the extensive body of science on neonicotinoid insecticides available at the time. In their review of aquatic ecotoxicology, (Pisa et al. 2015; Van der Sluijs et al. 2015; Pisa et al. 2017) they concluded that realistic levels of water contamination could lead to deleterious effects on the physiology and survival of a wide range of species in terrestrial, freshwater, and marine habitats. Chagnon et al. (2015) extended this analysis, suggesting that declines in emergent invertebrate prey due to insecticide use could plausibly cause population declines in insectivorous bird species.

Morrissey et al. (2015) conducted the first broad-scale quantitative risk analysis by comparing literature-based effect benchmarks with the growing body of information on residue levels in water bodies. They found that 81% of maximum and 74% of average individual neonicotinoid concentrations exceeded their benchmarks of 200 ng/L (acute) and 35 ng/L (chronic). They emphasized that the situation was likely worse because several neonicotinoids are often detected together, necessitating a comparison of summed concentrations with effect benchmarks. They concluded that both shortterm and long-term impacts of neonicotinoids were occurring on a broad geographical scale.

Sanchez-Bayo et al. (2016) reached similar conclusions, stating:

 "*Negative impacts of neonicotinoids in aquatic environments are a reality. . . . The decline of many populations of invertebrates, due mostly to the widespread presence of waterborne residues and the extreme chronic toxicity of neonicotinoids, is affecting the structure and function of aquatic ecosystems. Consequently, vertebrates that depend on insects and other aquatic invertebrates as their sole or main food resource are being affected*." (Sanchez-Bayo et al. 2016)

The most recent global analysis appears to be by Wang et al. (2022). They derived both acute and chronic benchmarks by generating species sensitivity distributions, combining toxicity data from all available aquatic taxa (algae, amphibians, crustaceans, fish, insects, molluscs, and worms). Their plotted values ranged over about six orders of magnitude. When chronic data were insufficient for a distribution, they used acute-chronic ratios to derive chronic toxicity data, a method we also employed in our earlier report (Mineau and Palmer 2013). While including all taxa increases data availability, it overlooks the different mechanisms of toxicity across groups, making it inappropriate to include them on the same plot. Nevertheless, their results are presented in Table A.8. Their ecosystem-wide  $\rm{HC}_5$  values under-protect sensitive groups like crustaceans and insects. Possibly for this reason, they recommend applying a safety factor of 5 to derive benchmarks from sensitivity distributions, a common practice among European regulators.



Although the principle of a single, all-encompassing toxicity distribution as performed by Wang et al. (2022) has significant limitations, an interesting takeaway from this benchmark derivation is the similarity in the chronic benchmark among all but one compound, all within a factor of 3. The proposed value of 5.9 ng/L for imidacloprid is very much in line with existing European benchmarks (Table A.3 above), although the methodology is completely different. Thiacloprid stands out as much more toxic than the others. When comparing their proposed benchmarks with measured water concentrations reported globally, they found no acute risks (unsurprising since their method under-protects), but chronic risks were often exceeded, with thiacloprid and acetamiprid predicted to have the greatest impact, followed by imidacloprid, clothianidin, and thiamethoxam. Only dinotefuran was predicted to present a "moderate" risk to aquatic ecosystems.

## **B.1 Monitoring and study results continue to show broad contamination of the aquatic environment**

EPA's 2016 review of imidacloprid (USEPA 2016) concluded that its levels frequently exceed thresholds at which aquatic invertebrate species are negatively impacted. The review indicated that several key taxonomic groups of aquatic invertebrates, not merely the most sensitive ones, are likely to be adversely affected by the concentrations currently measured in the environment. This concern is amplified by the frequent presence of other neonics in the same samples. EPA wrote:

 "*The risk findings for freshwater aquatic invertebrates do not depend solely on the high acute and chronic sensitivity of mayflies to imidacloprid. Rather, acute and chronic EECs exceed toxicity values for species distributed among multiple taxonomic groups of aquatic invertebrates.*" (USEPA 2016)

This conclusion was based on both effect levels and predicted exposures—the two key components of a risk assessment. EPA scientists were encouraged by the fact that actual water measurements closely matched their modeled levels. They estimated that 60% of seed treatment applications, 90% of soil applications, and 100% of foliar applications of imidacloprid would result in surface water contamination levels exceeding the 10.0 ng/L benchmark.

Morrissey et al. (2015) summarized global data, demonstrating that aquatic contamination is inevitable given current usage patterns and the sheer volume of neonics in use. The following examples highlight some key studies published since then:

Contamination of wetlands is expected and can be "excused" when applications are directly into the wetland or onto seasonally drained areas. Evelsizer and Skopec (2018) reported high contamination in field crops in Iowa, while Hayasaka et al. (2019) found similar results in Japanese rice paddies. Samson-Robert et al. (2014) detected levels as high as 55,700 ng/L of clothianidin and 63,400 ng/L of thiamethoxam in puddles on seeded fields, posing clear risks to aquatic organisms in these seasonal wetlands and indicating significant exposure for both vertebrate and invertebrate wildlife.

The persistence and solubility characteristics of neonics, however, coupled with their extensive use in a variety of conditions, have resulted in widespread environmental contamination. Anderson et al. (2013) found levels as high as 225,000 ng/L of thiamethoxam in playa lakes in North Texas. Main et al. (2014) reported clothianidin values up to 3,100 ng/L and thiamethoxam values up to 1,490 ng/L in small wetlands near canola seed treatments. Schaafsma et al. (2015) measured up to 16,200 ng/L of clothianidin and 7,500 ng/L of thiamethoxam in ditches outside cornfields and 3,250 ng/L of clothianidin and 16,500 ng/L of thiamethoxam in puddles up to 100 meters from the fields. In a later study, Schaafsma et al. (2019) observed maximum concentrations of 6,950 ng/L of clothianidin and 2,630 ng/L of thiamethoxam in tile drain water, with median concentrations of 350 ng/L and 680 ng/L, respectively, in water receiving tile drain inputs. These findings were from fields with an estimated application rate of only 19 g/ha of active ingredient.

Miles et al. (2017, with a 2018 correction) detected clothianidin concentrations as high as 450–670 ng/L in small lentic woodland bodies of water in Indiana, far from monitored corn and soybean fields. These levels were higher than those reported in ditch samples nearer to the fields. Cavallaro et al. (2019) reported values as high as 35 ng/L of clothianidin and 230 ng/L of thiamethoxam in wetlands within the canola-growing area of Saskatchewan, Canada.

Several studies have reported contamination levels far above benchmark levels early in the season, before any neonic use. For example, Schaafsma et al. (2015) found the highest levels pre-seeding, indicating year-round contamination. Extending exposure periods increases the risk of adverse effects, as toxicity is known to increase with longer exposure durations. Current assessments do not account for this, as chronic ecological impact studies typically last only a few weeks, whereas field data show that wildlife exposure periods span months to years. This prevents recovery of affected systems. Additionally, sublethal effects such as feeding disruption, behavioral changes, and delayed development have not been fully considered in the ecological assessments of neonics.

In previous reports, water monitoring data for New York State (Mineau 2019) and California (Mineau 2020) revealed frequent exceedances of aquatic toxicity benchmarks. Hoyle and Code (2016) arrived at similar conclusions. However, these analyses often miss the critical information of repeated exceedances at many sampling sites, crucial to understanding the full impact of neonics. This point was emphasized in Mineau and Palmer (2013) by reorganizing data from Starner and Goh (2012) in California watersheds. Mineau (2020) provided another example from California, showing that imidacloprid concentrations in Quail Creek between May and November seldom dipped below 500 ng/L, 50 times the 10 ng/L benchmark. This report shows the same pattern of persistence throughout the sample period—typically ice-free periods of spring to autumn. It is not surprising that there are increasing reports linking neonics to field impacts.

There is now incontrovertible evidence that pesticide loadings are a key factor in determining stream quality, as indicated by the presence of sensitive macroinvertebrates such as mayflies, caddisflies, and aquatic beetles (Reiber et al. 2020, Liess et al. 2021). Neonics, as the most important class of insecticides, significantly contribute to the degradation of freshwater systems worldwide and, likely, to estuarine and inshore marine environments. Associating specific compounds like neonics with biological outcomes such as insect emergence is challenging due to the natural variability and difficulty in obtaining sufficient replicates in aquatic field studies. Despite these methodological challenges, evidence is accumulating that neonics are having clear negative impacts on aquatic ecosystems, paralleling documented effects in terrestrial systems.

## **B.2. Increasing evidence of reduced insect biomass and emergence as a result of neonic contamination**

In Mineau and Palmer (2013), we reviewed an unpublished MSc thesis by Van Dijk (2010) from the Netherlands, which linked neonicotinoid contamination to reduced invertebrate numbers in Dutch canals. This work was later published as Van Dijk et al. (2013). Although Vijver and Van den Brink (2014) criticized the study for not accounting for other pesticide residues in the watersheds, Hallmann et al. (2014) indirectly supported Van Dijk's findings. They demonstrated that insectivorous birds declined in response to neonic concentrations (specifically imidacloprid) in water, and these declines did not occur before the introduction of neonics, despite the presence of other insecticides. Hallmann et al. (2014) predicted that regional bird declines would begin at water levels of imidacloprid of 200 ng/L or higher.

It is worthwhile to revisit Hallmann et al. (2014) in light of the continuing debate over benchmarks and safe levels in water (see Appendix A). The following figure (Figure B.1) is extracted from the article.





Clearly, if ecosystem-wide impacts are to be avoided, a chronic benchmark close to 10 ng/L is indicated. It is rather encouraging (even though the evidence of environmental damage is disturbing) to see such concordance between laboratory-derived benchmarks and ecosystem-wide impacts.

Nowell et al. (2017) showed a relationship between mayfly abundance and maximum imidacloprid concentrations in streams in the Midwest. Yamamuro et al. (2019) documented the collapse of a smelt fishery in Japan due to neonic contamination from rice paddy culture, with spring plankton populations declining by 83% and the smelt harvest dropping from 240 to 22 tons. In June 2018, the total neonic concentration in a lake tributary was 72 ng/L, with imidacloprid, clothianidin, and thiamethoxam detected following rice planting.

Cavallaro et al. (2019) emphasized that agricultural landscapes already subject wetlands to various pressures, such as fertilizer and sediment runoff, which affect aquatic quality. They found that neonic inputs (primarily clothianidin and thiamethoxam, but also imidacloprid and acetamiprid) impacted insect emergence, habitat quality, and diversity. Their results showed that 73% of samples contained mixtures of neonics from different canola treatments.

Schepker et al. (2020) surveyed 26 wetlands in Nebraska during the spring of 2015, coinciding with the waterfowl spring migration rather than agricultural activities. They detected imidacloprid (max 5 ng/L) and/or clothianidin (max 16 ng/L) in 85% of wetlands, despite levels being below EPA benchmarks. They found that a buffer of more than 50 meters around wetlands reduced insecticide concentrations, but even at low levels, total neonic concentration negatively affected nektonic biomass.

Barmentlo et al. (2021) conducted an experiment with biweekly spikes of thiacloprid (100 to 10,000 ng/L) in ditches. Dragonflies, damselflies, and caddisflies showed reduced emergence following two 100 ng/L spikes, and total biomass and diversity were affected at 1000 ng/L. Over 30 days, the two 1000 ng/L spikes equated to a time-weighted concentration of 300 ng/L. The authors noted that changes in individual species often masked the broader disruptions caused by the insecticide, with some species benefiting from competition release as more sensitive species were impacted. They highlighted that these changes occurred at neonic levels commonly recorded worldwide and that they had likely underestimated the full impact due to the short study duration.

The work of Schmidt et al. (2022) in California showing current impacts on mayfly populations was reviewed earlier. In an ideal world, the good correspondence between laboratory-based predictions and the field should encourage regulators to impose more stringent regulatory benchmarks as well as restrictions and cancellations to reduce the environmental impact. This is clearly what has happened in Europe. In the United States and Canada, regulators appear to have paid no attention to the accumulating evidence. This evidence clearly underscores the significant impact of neonicotinoids on insect biomass and emergence. This, in turn, affects higher trophic levels, including insectivorous birds, thereby indicating a broader ecological disruption linked to neonic contamination.

## **B.3. Challenges in routine water monitoring of neonicotinoid levels**

Routine water-monitoring exercises often fail to detect neonicotinoid levels as high as those reported in scientific literature. This discrepancy arises because data from broad water-monitoring programs typically rely on "grab samples," which can significantly underestimate peak surface concentrations of pesticides. Xing et al. (2013) demonstrated that relying on grab samples can lead to an underestimation by several orders of magnitude. This issue has been echoed by other researchers, such as Barmentlo et al. (2021), who emphasize the inadequacy of grab sampling in capturing peak pesticide concentrations.

The necessary frequency of sampling should be determined on the basis of the watershed's size, as suggested by Crawford (2004). Crawford estimated that during runoff periods, samples should be taken at least 10 times monthly to ensure that peak measured residues are within a factor of 2 of the likely maxima. However, many monitoring sites, such as those in Minnesota, do not meet this criterion. This significant issue is often overlooked by regulators and state or watershed authorities, who typically focus on reporting the fraction of samples that exceed benchmark values without addressing the limitations of their sampling methodologies.

Accurate assessment of neonicotinoid contamination requires more frequent and methodologically sound sampling practices to capture true peak concentrations, thereby providing a more realistic picture of environmental exposure and risks.

#### B.3.1. The interpretation of measured water concentrations

USGS, recognizing the problems of occasional sampling as well as the problem of detection limits leading to heavily censured datasets, designed a sophisticated modeling approach (the SEAWAVE-QEX model—Vecchia 2018). The model may be difficult to use below 10 sampling visits per site,<sup>16</sup> which may rule out its application for several of the sampling sites in Minnesota.

Figure B.2 is taken from Vecchia (2018) and shows the concentration of carbaryl in the Kisco River in New York State, with a detection limit just above 0.01 ug/L and peak residue detections around 0.1 ug/L. Estimated annual maximal values are commonly three to four times the highest observed value and sometimes more than 10 times the measured values.

**Figure B.2. Plot taken from Vecchia (2018) to show the relationship between observed concentrations, simulated concentrations as a result of analyses below detection levels, and the estimated yearly maxima for carbaryl concentrations in the Kisco River, NY.**



16 The full requirements are stated as follows: at least three individual years with six or more observations, 30% or more of which are uncensored; at least 30 observations for all years combined; and at least 10 uncensored observations for all years combined.

USEPA is currently considering how this model can be used in its drinking water assessments (USEPA 2020b and subsequent reviews by the Science Advisory Panel) but, to my knowledge, has not proposed applying any correction to water sampling data in order to assess ecological impacts.

Another limitation of most current datasets is that sampling is restricted to the summer months. Although this does cover most of the season of use of the pesticides, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP 2020), in its guidance to EPA, points out that "*in some situations, winter storms, especially the first flush after the dry period, often generate peaks in pesticide concentration.*"

When it comes to using grab water samples to perform an ecological risk assessment, it is clear that using the raw data is fraught with problems and gives a false sense of security while under-protecting receiving environments. I believe we can demand better from EPA and other regulatory bodies.

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