# NEONICOTINOIDS ON THE LANDSCAPE: EVALUATING AVIAN EXPOSURE TO TREATED SEEDS IN AGRICULTURAL LANDSCAPES

Charlotte Roy, Da Chen[1], Julia Ponder[2], Mark Jankowski[3]

## SUMMARY OF FINDINGS

Neonicotinoid pesticides (e.g., imidacloprid, thiamethoxam, thiacloprid, clothianidin) are commonly applied to agricultural seeds (e.g., corn, soybean, wheat, sunflower), and are known to cause lethal and sub-lethal effects in birds. Neonicotinoid-treated seeds could be available to wildlife through spillage or exposure to treated seeds near or at the soil surface after planting (de Leeuw et al. 1995, Pascual et al. 1999, Lopez-Antia et al. 2016). We are examining sub-lethal exposure of wild birds to these pesticides in agricultural landscapes of Minnesota. We are quantifying seed availability at the soil surface in recently planted fields and the rate of seed spills during planting, as well as documenting birds eating treated seeds through field studies with trail cameras and harvested birds. Thus far, we have documented ring-necked pheasants (*Phasianus colchicus*), Canada geese (*Branta canadensis*), American crows (*Corvus brachyrhynchos*), various species of sparrows (Emberizidae) and blackbirds (Icteridae), as well as white-tailed deer (*Odocoileus virginianus*), rodents, leporids, and raccoons (*Procyon lotor*) consuming seeds. In 2016, we documented 212 seed spills in 38 townships during planting but missed the peak of planting in many of the townships we surveyed. We documented exposed seeds at the soil surface in plots at 25% of 48 fields sampled after planting in 2016. Field work is ongoing for 2017. We are still conducting analyses to determine the length of time that neonicotinoids persist on seeds exposed at the soil surface, and whether the seeds are consumed before the chemicals have degraded.

We also conducted laboratory experiments to try to identify non-lethal sampling methods that could lead to methods for measurement of individual and population-level exposure, including residues in excreta and blood. Residues were highest (geometric mean) in the brain, followed by liver, spleen, muscle, blood, kidney, then feces in birds dosed in the lab. Residues were detected in 90.9% of domestic chicken fecal samples collected in the lab, the highest detection frequency of all tissues tested. Forty-one of 46 (89%) liver samples collected from hunter-harvested sharp-tailed grouse (*Tympanuchus phasianellus*) and 18 of 27 (67%) hunter-harvested greater prairie-chickens (*Tympanuchus cupido*) have been analyzed and contained detectable concentrations of at least 1 neonicotinoid. Similarly, 22 of 34 (65%) fresh prairie-chicken fecal pellets and 47 of 56 (84%) sharp-tailed grouse pellets collected from leks have been analyzed and had detectable concentrations of at least 1 neonicotinoid. Data collection will continue through fall 2017.

[1] Southern Illinois University Carbondale (SIUC)
[2] University of Minnesota, College of Veterinary Medicine (UMN CVM)
[3] Environmental Protection Agency (EPA)

## INTRODUCTION

Neonicotinoids are the most widely used pesticides worldwide (Mineau and Palmer 2013), comprising 25% of the global agricultural chemical market. Their action is highly specific to invertebrates, with comparatively low toxicities for vertebrates compared to pesticide options predating the early 1990s (Tomizawa and Casida 2005, Jeschke et al. 2011). This high specificity contributed to their widespread and rapid adoption, beginning in 1994 with the registration of imidacloprid in the United States.

Recently, neonicotinoids have received a lot of attention because of their potential toxicity to bees and other pollinators, and their possible role in colony collapse disorder. Several neonicotinoid treatments were banned or placed under a moratorium in Europe in 2013, and neonicotinoids are currently under registration review by the Environmental Protection Agency (EPA) in the United States. The Minnesota Department of Agriculture (MDA) is currently reporting a process and criteria for review of neonicotinoid use with an emphasis on pollinators (MDA 2014). However, recent concern has not been limited to pollinators; the American Bird Conservancy called for research on the effects of neonicotinoids on birds and a ban on neonicotinoid seed treatments (Mineau and Palmer 2013). Evidence is accumulating that vertebrates are also adversely affected by these pesticides (see reviews in Mineau and Palmer 2013, Gibbons et al. 2014). MDA (2014) acknowledged that, "Although neonicotinoids are less toxic to vertebrates than to arthropods, direct consumption of neonicotinoid treated seeds may expose birds and other taxa to acute or chronic doses."

The most likely route of exposure to large doses of neonicotinoids for birds is ingestion of treated seeds (Goulson 2013, Gibbons et al. 2014), although numerous other mechanisms exist (e.g., soil, trophic transfer; SERA 2005, Douglas et al. 2015). Ingestion of a small number of neonicotinoid-treated seeds is lethal to birds; for example, a single treated corn kernel can kill a blue-jay sized bird (see reviews in Mineau and Palmer 2013, Gibbons et al. 2014). However, toxicity generally varies by chemical and species, given differences in physiological make-up such as size and digestive processes. Lethal impacts are rapid and difficult to detect in the wild although a few pesticide poisoning incidents have been detected (Greig-Smith 1987, Fletcher et al. 1995, Berny et al. 1999, de Snoo et al. 1999). Sub-lethal exposure might be easier to detect in the wild. Sub-lethal effects in birds in the lab include hyporeactivity, lack of coordination, wing drop, immobility, eggshell thinning, reduced egg hatching rate, impaired testicular function, immune suppression, and low weight in chicks (Cox 2001, Lopez-Antia et al. 2013 and 2015, Tokumoto et al. 2013, Mineau and Palmer 2013). Reproduction can be affected by consumption of just 1/10$^{th}$ of a treated corn seed per day during egg-laying (Mineau and Palmer 2013).

Thirty bird species were observed picking up treated seeds from cereal fields in Spain and 3.1% of partridge gut contents collected by hunters tested positive for imidacloprid after planting of winter cereal crops (Lopez-Antia et al. 2016). Dead and poisoned partridges have been found in agricultural fields in France following use of imidacloprid-treated seed (Berny et al. 1999). The EPA estimated that ~1% of seeds remain accessible to granivores after planting (as reported by Goulson 2013, Lopez-Antia et al. 2015). Unfortunately, neonicotinoid use of "treated articles," such as seed, is not currently tracked by the government due to the exemption in 40CFR §152.25(a). Yet, almost all corn planted in the Midwest has been treated with these pesticides (Stokstad 2013), as well as most soybean, wheat, and sunflower seeds, and they are widely used with other application methods for other crop types.

Studies of neonicotinoid effects on vertebrates are overwhelmingly laboratory-based (91% of studies), which limits our ability to interpret the significance of findings in more natural settings (Gibbons et al. 2014). Higher densities of exposed seeds result in greater attraction of birds to

fields (Murton et al. 1963, Feare et al. 1974). Bednarska et al. (2013) identified a need for feeding rate information in the field to allow extrapolation of lab data to the field. Lopez-Antia et al. (2013) pointed to a "need for evaluation of real exposure to coated seed ingestion by wild birds, including feeding behavior analyses and estimation of food intake rates." We are therefore conducting a study to develop tools with which we are ascertaining whether birds are at risk for exposure to neonicotinoid-treated seeds in agricultural landscapes.

## OBJECTIVES

The overarching objective is to ascertain whether birds are at risk for exposure to neonicotinoid-treated seeds in agricultural landscapes. Specifically, we will:

1. Identify birds consuming neonicotinoid-treated seeds and quantify consumption per foraging bout.
2. Quantify the rate of seed spillage and surface seed exposure after planting within fields.
3. Quantitatively link exposure and tissue/blood/excreta to neonicotinoid concentrations in chickens (lab study).
4. Determine whether neonicotinoid exposure in wild prairie grouse can be detected from non-lethal sampling methods or from hunter harvested birds (pilot field study).

## METHODS

### Documenting Consumption of Treated Seeds

In 2016, we selected 12 Wildlife Management Areas (WMAs) to place trail cameras from the 1,707 WMAs in Minnesota, of which a subset have food plots or Cooperative Farming Agreements (CFAs). The available data on CFAs on DNR-managed land indicated 7,420 acres (3,003 ha) of row crops in 341 CFAs in Region 4 (southern region) and 2,431 acres (984 ha) of row crops in 66 CFAs in Region 1 (northwest region; M. Benage and J. Williams, respectively, pers. comm.). We selected WMAs with a land cover composition similar to that of the surrounding landscape using the 2014 National Cropland Data Layer (USDA-NASS 2015) in ArcGIS 10.2 (ESRI 2015), but required them to have food plots or Cooperative Farming Agreements (CFAs) after they met the first criterion. Working on WMAs minimized bias in farming activities that might result from prior knowledge of the study. Furthermore, neonicotinoid-treated seed has been commonly used by private farmers on WMAs and many of the managers reported difficulty finding seeds that had not been treated. We prioritized this portion of the study in 2016 because farmers were prohibited from planting neonicotinoid-treated seeds on WMAs beginning in 2017.

Cameras were placed to minimize risk of theft and to view a recently planted field to document foraging at a simulated seed spill and exposed or submerged seeds or seedlings. Spills were simulated with 1000 corn or soybean seeds to allow determination of the time for birds to discover spills and the number of seeds consumed in each foraging bout/bird. Additionally, we placed cameras at 2 privately owned fields. Cameras were deployed in each location for 3–6 weeks after planting. At each field, 2 cameras were deployed; one that captured 1 image/sec in still photos and a second that captured 60 sec of video when triggered by motion. The camera set for still photos also took field scans at 5 min intervals between 0600–0800 hr and 1830–2030 hr to document birds foraging in fields during sunrise and sunset periods during the planting season. Images are currently being examined to identify species, number of birds consuming seeds, and number of seeds consumed per foraging bout, or in broader views, to document birds using crop fields after planting.

In 2017, we included more privately owned fields, which were generally larger than fields planted on WMAs. We placed 1 camera at each of 24 privately-owned fields in addition to placing cameras at 16 WMAs. Instead of capturing still images at simulated spills, which often

produced ambiguous information about whether seeds were ingested, we instead set the cameras to record video only. In 2017 we also simulated spills with wheat, in addition to corn and soybean. We checked cameras once weekly to replace batteries and data cards and deployed cameras in each location for 2 weeks. Data collection was still underway at the time this report was written.

### Quantifying Spills and Seed Surface Exposure

All chemically treated seeds (e.g., neonicotinoids, fungicides, other pesticides) are unnaturally colored, as mandated by the Federal Seed Act. These seeds are highly visible and easily identified by their unusual color (e.g., pink, blue, green, purple), which is used to prevent accidental feeding to livestock. We quantified the frequency of seed spills on the landscape by inspecting fields with visual access from roads, field access points, and roadsides in agricultural areas. We hoped to avoid bias in spill rates that might result from obtaining permission to access privately owned fields on foot, but this method makes the implicit assumption that spill rates associated with refilling hoppers and overfilling is similar for fields adjacent to roads and fields that are not adjacent to roads.

We identified 211 townships in the western third and southeastern part of the state with at least 50 miles of roads and 50% of the area in corn, soybeans, and/or wheat production using the 2014 Cropland Data Layer (USDA-NASS 2015) and the Department of Transportation (DOT) Roads Layer (DOT 2008) in ArcGIS. These criteria were used to select townships with visual access to fields from roads, while also not becoming so restrictive that the spatial distribution of the sample was constrained. We drew a spatially balanced sample of 50 townships and surveyed the 38 most western townships selected due to a later start to planting during the spring of 2016. In 2017, we selected 50 different townships and again surveyed the 38 westernmost townships due to a late start to planting, for a total of 76 townships surveyed during the 2 years of the study. We began in the southern counties and worked north beginning in late April as crops were planted.

We recorded locations and approximate number of seeds in spills near *recently planted* fields with the DNRSurvey mobile computer application. Documenting only *recently planted* fields allowed for control in temporal variation in the timing of planting. For example, a field that has not been planted yet will not have a spill at the time of sampling, which is different from a spill not occurring during planting. Thus, by only including recently planted fields in our estimates, we measured spills during planting. We defined a "field" as a quarter quarter-section (i.e., 40 acres). We recorded each quarter quarter-section in agricultural production, whether any part of it was recently planted (i.e., $\leq$early seedling stage), documented the amount (number of seeds) of spilled seed on the road, field edge, or visible in the field, and crop type (when possible). To determine the proportion of seed spills that contain neonicotinoid-treated seed, we collected seeds from accessible spills and will quantify 7 neonicotinoids (Chen et al. 2014).

To estimate the amount of seed at the soil surface after planting, we used a 1-$m^2$ frame to define plots in recently planted fields and counted all treated seeds visible within the frame after planting (Lopez-Antia et al. 2016). We sampled 5 plots in a field corner and 5 plots in the field center as estimated visually from field boundaries while standing in the field. For corner locations, we randomly selected 1 field corner per field by flipping a coin twice, and paced 15 m and 30 m along each edge in an L-shape that had the field corner for a vertex for a total of 5 measurements. This approach incorporated sampling parallel and perpendicular to planting rows, and we suspected that seed exposure would be greater at the end of rows at turning points than within rows. For field centers, we paced 15 m in each cardinal direction to sample for a total of 5 measurements including the center.

### Linking Exposure to Concentrations in the Lab

We are quantitatively linking field sample concentrations to lab exposure concentrations through work with University of Minnesota - College of Veterinary Medicine (UMN-CVM) and Southern Illinois University Carbondale (SIUC). We are determining how many days post-exposure imidacloprid (i.e., the most common seed treatment in Minnesota, J. Zachmann, MDA, pers. comm.) is detectable in both non-lethally and lethally collected samples. A non-lethal method to determine sub-lethal exposure would facilitate data collection during spring planting when spills would be expected to be most numerous.

At UMN, domestic chickens (*Gallus gallus domesticus*) were orally exposed to imidacloprid (IMI) for 7 days and serially sampled during and after the course of exposure to simulate repeated sub-lethal exposures. Chickens served as our model species given their suitability to captivity and close taxonomic relationship with wild grouse (Family Phasianidae). Small sample sizes are commonly used in dosing studies because the differences among treatment groups are expected to be very large and variability within groups low (e.g., Berny et al. 1999, Bednarska et al. 2013). We exposed animals (n = 5) to 1, 5, and 20% of the $LD_{50}$ (104.1 mg/kg IMI, Kammon et al. 2010) daily for 7 days by giving ~1.5 kg birds a daily IMI bolus of 1.04 mg/kg/day ("low"), 5.20 mg/kg/day ("medium"), and 20.80 mg/kg/day ("high"). The $LD_{50}$ is the single dose that is expected to be lethal to 50% of test subjects. The $LD_{50}$ could be obtained if chickens ingested ~260–946 corn seeds (depending on application rate to seeds, which varies among seed companies), or stated differently, 3–10 seeds is comparable to the 1% $LD_{50}$ dose. Thus, these were realistic doses. Prairie grouse are smaller (0.6–1.2 kg) and thus a smaller dose (104–780 seeds depending on bird weight) would be expected to produce similar results. Other neonicotinoids have a lower $LD_{50}$ than IMI so lethality would be expected at much lower seed ingestion levels than for IMI.

The full experiment was completed only for birds in the low and medium treatment groups, as birds in the high group were humanely euthanized on day 1 due to severe neurological and respiratory depression. Prior to exposure, baseline blood and excreta samples were collected. Sequential blood and excreta samples were collected on experiment days 1–21. Blood samples were collected at 0, 8, and 24 hours post-exposure, and then on days 8, 14, and 21 post-exposure. Birds that were considered at endpoint and euthanized had blood samples taken immediately before euthanasia. The low group was sampled for feces 1 day earlier than the medium group due to logistical challenges. Internal organ (i.e., brain, kidney, liver, spleen) and muscle samples were taken from birds that died during the treatment period or on day 21, whichever came first. Birds were weighed on all days of sampling. Samples were sent to SIUC for residue analysis (Chen et al. 2014).

Descriptive statistics and graphing of the available data from these lab studies was performed to understand in a preliminary sense how IMI concentrations changed over time, and in response to dose, on a tissue-specific basis. According to best practices, we have used geometric rather than arithmetic mean for chemical concentration data, which are typically lognormally distributed. Arithmetic mean is often biased high. Further statistical analyses will be conducted once the full dataset, including metabolites (i.e., neonicotinoids modified through metabolic processes), is obtained.

### Detecting Neonicotinoids in Free-Ranging Birds

We also collected samples from wild birds through both invasive and non-invasive methods to try to identify ways to assess exposure to neonicotinoids in the field. Fresh fecal pellets and blood samples from trapped prairie grouse were collected during lek visits for a genetic study in spring 2015. Samples were stored frozen until shipped to the lab at SIUC. Hunters also voluntarily submitted harvested prairie grouse in fall 2015. Tissues and fecal pellets are

being tested for thiacloprid (THIA), acetamiprid (ACE), thiamethoxam (TMX), imidacloprid (IMI), clothianidin (CLO), dinotefuran (DIN), and nitenpyram (NTP).

MNDNR staff also assisted with collections of birds observed foraging on treated seeds in the spring of 2016 under federal permit MB682323-0 issued to MNDNR. We are examining exposure from ingesta and tissue residue levels according to Chen et al. (2014) at SIUC.

## RESULTS

### Documenting Consumption of Treated Seeds

We are still viewing images collected by trail cameras at simulated spills during spring 2016 (*n* = 188,399 photos and 12,602 videos). In the images viewed to date, we have documented ring-necked pheasants, Canada geese, American crows, various species of sparrows and blackbirds, white-tailed deer, rodents, lagomorphs, and raccoons consuming treated seeds. We will continue viewing images during fall 2017 and winter 2017–18 and summarize results in future research summaries.

### Quantifying Spills and Seed Surface Exposure

We observed 212 large seed spills that were visible from the road during surveys of 38 townships during 2016. However, we missed the peak of planting in many of the townships surveyed because the spring of 2016 was very wet and crops were planted later than usual. Nevertheless, at the time of our road-based surveys, 79,386 acres of corn, 82,341 acres of soybeans, 69,293 acres of wheat, and 7,753 acres of other crops were planted in the areas surveyed. Spill rates in the areas surveyed were calculated as 4 spills/10,000 ac corn, 14 spills/10,000 ac soybeans, 7 spills/10,000 ac wheat, and 15 spills/10,000 ac other crop types. Extrapolating statewide requires the assumption that spill rates visible in fields adjacent to roads are representative of spill rates in fields located elsewhere. If spills near roads are more likely to be cleaned up than those less visible to passersby, then this assumption may not be tenable. Yet, we did not observe spills being cleaned up during our surveys. Furthermore, most spills occur during hopper refilling, and this often occurs near field access points along roads. Thus we think our assumptions are reasonable. Applying our spill rates across the acres farmed statewide (8,450,000 acres of corn, 7,550,000 acres of soybeans, and 1,321,000 acres of wheat were planted in Minnesota during 2016 (National Agricultural Statistics Service; last accessed 5 June 2017 National Agricultural Statistics Service), we estimate nearly 15,000 large seed spills statewide and expect that if there is a bias, our estimates are biased low.

We documented exposed seeds at the soil surface in 25% of the 48 fields where we sampled 1 $m^2$ plots in 2016. Seeds were exposed in $\geq$1 centrally located plot in 14.6% of fields measured. Exposed seeds were detected in $\geq$1 corner plot of 18.8% of fields measured. Most (79%) of the fields we measured were planted to corn, 17% were planted to soybeans, and 4% were planted to wheat. Most (96%) sampled fields were on public land but 79% of the sampled fields on public land were planted by private cooperating farmers with their own equipment. We suspect that spill rates are influenced by the type of equipment used for sowing (Lopez-Antia et al. 2016) and possibly the seed type. These numbers are considered preliminary and subject to future revision. Data for the 2017 field season will be included in future reports.

### Linking Exposure to Concentrations in the Lab

We collected 72 blood samples, 100 fecal samples, 15 muscle, brain, liver, and kidney samples, and 103 eggs during experiments for neonicotinoid analysis. Imidacloprid (IMI) was detected more frequently and for a longer duration post-exposure in fecal samples (90.9%, $\leq$21 days post exposure) than blood (32.9%, $\leq$7 days post exposure). Blood concentrations increased from the

first samples taken at the start of the experiment (hr 0), increased at hr 8 and declined again at hr 24 (Figure 1); after this time, samples did not contain detectable IMI except for 1 sample taken on day 8. Fecal IMI concentrations followed a 3rd order polynomial pattern, increasing from the start of the experiment (day 0) until approximately day 6, decreasing until day 18 and holding steady or slightly increasing by day 21 (Figure 2). The low dose group tended to exhibit lower IMI fecal concentrations than birds in the medium dose group, as expected (Table 1). IMI was rapidly removed from blood, but the change in concentrations varied 17,234-fold (c.f., 279-fold in feces; fold change is maximum detected concentration/minimum detected concentration across all groups and times), and thus blood may provide a more sensitive indicator of an acute exposure than feces. By contrast, fecal samples provided a more integrated, longer, and more consistent detection in exposed birds (Figure 2) and thus may be more applicable to field applications where time from chemical exposure will be more variable.

IMI was measured in internal organs (Figure 3) collected on the final day of the experiment, depending on when birds were euthanized. Low- and medium-dosed birds were euthanized on day 21, whereas high-dosed birds were euthanized after showing clinical signs of distress on day 1. Detection frequency of IMI was highest in kidney, liver, and spleen (73.3%), although muscle and brain also exhibited similar detection frequencies (66.7%). Geometric mean tissue concentrations were highest in brain and lowest in the kidney (Table 2).

### Detecting Neonicotinoids in Free-Ranging Birds

Field-collected prairie grouse samples sent for neonicotinoid analysis included 61 sharp-tailed grouse fecal pellet groups and 34 greater prairie-chicken fecal pellet groups collected in 2015, and 46 and 27 pellet groups, respectively, in 2017. We also collected 5 blood samples from trapped sharp-tailed grouse, as well as 2 brains and 3 breast muscles from sharp-tailed grouse for which we had whole carcasses and sent them for neonicotinoid analysis. Hunters submitted livers from 11 prairie-chickens, 22 sharp-tailed grouse, and 3 prairie-chicken/sharptail hybrids during fall 2015, and 16 prairie-chicken, 26 sharp-tailed grouse, and 2 pheasant livers during fall 2016.

A subset of field samples from wild prairie grouse has been analyzed for neonicotinoids thus far. Forty-one of 46 (89%) livers collected from hunter-harvested sharp-tailed grouse, 18 of 27 (67%) greater prairie-chicken livers, and 3 of 3 sharptail-chicken hybrids from hunter-submitted samples had detectable concentrations of at least 1 neonicotinoid. Three of 3 blood samples analyzed thus far have tested negative for neonicotinoids. Dinotefuran and NTP were not detected in any samples. The most commonly detected neonicotinoids in prairie-chicken livers were IMI (63%), CLO (11%), THIA (4%), ACE (4%), and TMX (4%). The most commonly detected neonicotinoids in sharp-tailed grouse livers were IMI (83%), CLO (13%), THIA (13%), ACE (9%), and TMX (2%). Maximum concentrations of neonicotinoids in prairie-chicken livers were 8.3 ng/g IMI, 4.2 ng/g CLO, 1.1 ng/g THIA, 0.21 ng/g, ACE, and 0.43 ng/g TMX, respectively. Maximum concentrations detected in livers of harvested sharp-tailed grouse were 84.5 ng/g IMI, 3.58 ng/g CLO, 1.18 ng/g THIA, 0.71 ng/g ACE, and 0.5 ng/g TMX. Similarly, 22 of 34 (65%) fresh prairie-chicken fecal pellets and 47 of 56 (84%) sharp-tailed grouse pellets collected from leks during spring 2015 contained detectable concentrations of at least 1 neonicotinoid. The most commonly detected neonicotinoid in the greater prairie-chicken fecal pellets was IMI (71%), followed by CLO (9%), and THIA (9%). Acetamiprid and TMX were not detected in feces, perhaps due to differences in the way they are metabolized or excreted. Maximum concentrations of IMI, CLO, and THIA in feces were 6.12 ng/g, 0.90 ng/g, and 1.05 ng/g, respectively. In sharp-tailed grouse pellets, the most commonly detected neonicotinoids were IMI (80%), CLO (21%), THIA (11%), ACE (2%), and TMX (2%). Maximum concentrations were 39.7 ng/g IMI, 7.57 ng/g CLO, 0.9 ng/g THIA, 0.2

ng/g ACE, and 0.5 ng/g TMX. Samples which contained multiple neonicotinoids (n = 16 livers and 14 pellets) generally contained IMI, except for 4 livers and 2 pellets.

Birds collected while foraging on treated seeds included 1 ring-necked pheasant, 5 red-winged blackbirds (*Agelaius phoeniceus*), 2 yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), 4 brown-headed cowbirds (*Molothrus ater*), and 5 common grackles (*Quiscalus quiscula*). Two brown-headed cowbird livers tested positive for exposure to IMI and CLO. One yellow-headed blackbird liver tested positive for IMI. Livers of all other birds collected while foraging on treated seeds tested negative for recent neonicotinoid exposure.

## DISCUSSION

Fecal samples appear to provide a possible non-invasive means to detect exposure in birds based on our findings and the potential to refine analytical methods. Previous studies have demonstrated that neonicotinoids (e.g., thiamethoxam) are excreted primarily through the kidneys in mammals (Bednarska et al. 2013, Tomizawa and Casida 2005). Ongoing analytical work to measure metabolites of imidacloprid in feces is expected to provide a more sensitive (i.e., higher fold concentration change) assay than current parent compound (i.e., imidacloprid unmodified by metabolic processes) data. Further work will be required to quantify how the potential environmental imidiacloprid exposure scenarios (concentration, duration, and frequency) influence the detection of parent compound and metabolites in feces and the uric acid wash. However, fecal samples could be collected from the GI tract of hunter-killed birds, from live birds, or non-invasively from the environment. Further work is necessary to refine non-invasive collection because UV light can and microbial degradation may degrade neonicotinoids (Lu et al. 2015; Lu et al. 2016; Ma et al. 2014), so pellet freshness would be an important consideration.

Our data provide evidence that internal organs can serve as an indicator of imidacloprid exposure in lethal collections including hunter-killed birds. However, based on detection frequencies in organs and feces, fecal samples may provide a more reliable index of exposure than organs. Berny et al. (1999) reported that liver and kidney had the most consistent imidacloprid concentrations in fatally exposed wild birds, whereas crop and gizzard provided inconsistent concentrations. However, Lopez-Antia et al. (2015) reported that imidacloprid could be consistently detected in crops and livers of dosed partridges (*Alectoris rufa*).

The highest concentration of imidacloprid detected in livers of harvested prairie grouse was higher than that of chickens in the low and medium dose group at the end of the experiment. However, it was lower than the high $LD_{50}$ group after early euthanization. Similarly, the highest concentration of imidacloprid detected in field collected feces was lower than both the 1% and 5% dose groups shortly after exposure, and was more similar to both of these groups a few weeks post-exposure. We cannot know if this indicates a lower initial exposure, the passage of time since exposure, or both; but, given that 1% $LD_{50}$ (1.04 mg/kg) is comparable to the dose received after consuming 3–10 corn seeds and that imidacloprid can be detected in tissues at least 21 days post-exposure, we consider it likely that this finding reflects an exposure to imidacloprid that occurred a few weeks prior to sample collection. Winter wheat is planted in September and October in Minnesota, so grouse might be newly exposed to treated seeds in the fall, although it is not clear how long spring exposure would be detectable in organs. At a minimum, detection of imidacloprid in tissues of wild birds provides us with a qualitative index of exposure, which is one step closer to understanding the effects of imidacloprid in wild birds in Minnesota.

The high detection frequencies of imidacloprid in internal organs on experimental day 21 after 7 consecutive days of exposure indicates a persistence of imidacloprid that is notable but not easily comparable to other acute studies. Most studies have suggested a rapid metabolism and

elimination (~48 hours) of parent (i.e., unchanged) compound in the urine after *single* oral doses (Bednarska et al. 2013; Tomlin 2004). Our findings demonstrated a relatively high persistence of parent compound in feces and organs and may therefore indicate an appreciable toxicological risk for birds.

The locations of the compounds in the tissues provide insight into which systemic effects warrant examination. Based on the high splenic concentrations, we hypothesize imidacloprid will cause immune system changes in birds. The detection of imidacloprid in neurological tissues (brain) indicates a potential for behavioral changes as well. If immune system or behavioral effects impact survival and reproduction, then population–level impacts are plausible. Our laboratory data will be useful in understanding the absorption, distribution, excretion, and effects of imidacloprid, as well as in the design of future laboratory and field studies in birds. We will also contribute some of the first information on exposure of wild birds in the United States to neonicotinoids.

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Table 1. Summary of imidacloprid detections in domestic chicken blood and feces in each of 3 dose groups at University of Minnesota – College of Veterinary Medicine in 2015. Note that birds in the high dose group were euthanized early, which may have limited the ability to eliminate imidacloprid in feces.

| | Dose (mg/kg/day) | N | Percent detects | Fold change | Median | Geometric mean | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| Blood (ng/ml) | 1.04 | 6 | 20.0 | 4.2 | 1.7 | 1.4 | 0.5 | 2.1 |
| | 5.02 | 10 | 33.3 | 9.8 | 2.6 | 2.2 | 0.7 | 6.9 |
| | 20.80 | 8 | 61.5 | 2051.7 | 3270 | 805.6 | 4.2 | 8617 |
| Feces (ng/g wet weight) | 1.04 | 26 | 81.3 | 91.8 | 14.6 | 10.1 | 0.8 | 73.4 |
| | 5.02 | 39 | 97.5 | 278.9 | 19.1 | 14.1 | 0.7 | 195.2 |
| | 20.80 | 5 | 100.0 | 2.8 | 3.2 | 3.7 | 2.3 | 6.5 |

Table 2. Summary of tissue concentrations of imidacloprid in all laboratory-exposed chickens for all dose groups combined at University of Minnesota – College of Veterinary Medicine in 2015.

| Tissue | First detection (day) | Last detection (day) | Fold change | N | Percent detects | Min conc[a] | Max conc[a] | Median conc[a] | Geometric mean conc[a] | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| Feces | 1 | 21 | 279 | 70 | 90.9 | 0.7 | 195 | 14.6 | 11.3 | 35.9 |
| Kidney | NA[b] | NA | 1681 | 11 | 73.3 | 0.5 | 823 | 1.7 | 13.4 | 276.5 |
| Liver | NA | NA | 19882 | 11 | 73.3 | 0.3 | 5766 | 6.7 | 64.6 | 2473.6 |
| Spleen | NA | NA | 30413 | 11 | 73.3 | 0.2 | 6387 | 16.8 | 63.6 | 2320.8 |
| Brain | NA | NA | 10410 | 10 | 66.7 | 0.6 | 5725 | 1212.7 | 76.7 | 2295.8 |
| Muscle | NA | NA | 3469 | 10 | 66.7 | 0.8 | 2775 | 382.3 | 62.8 | 1128.5 |
| Blood | 1 | 8 | 17234 | 24 | 32.9 | 0.5 | 8617 | 4.1 | 14.1 | 2389.5 |

[a] Conc = concentration (ng/g wet weight in tissues and ng/ml for blood).
[b] NA = Not applicable because tissues were collected when chickens were killed the last day.

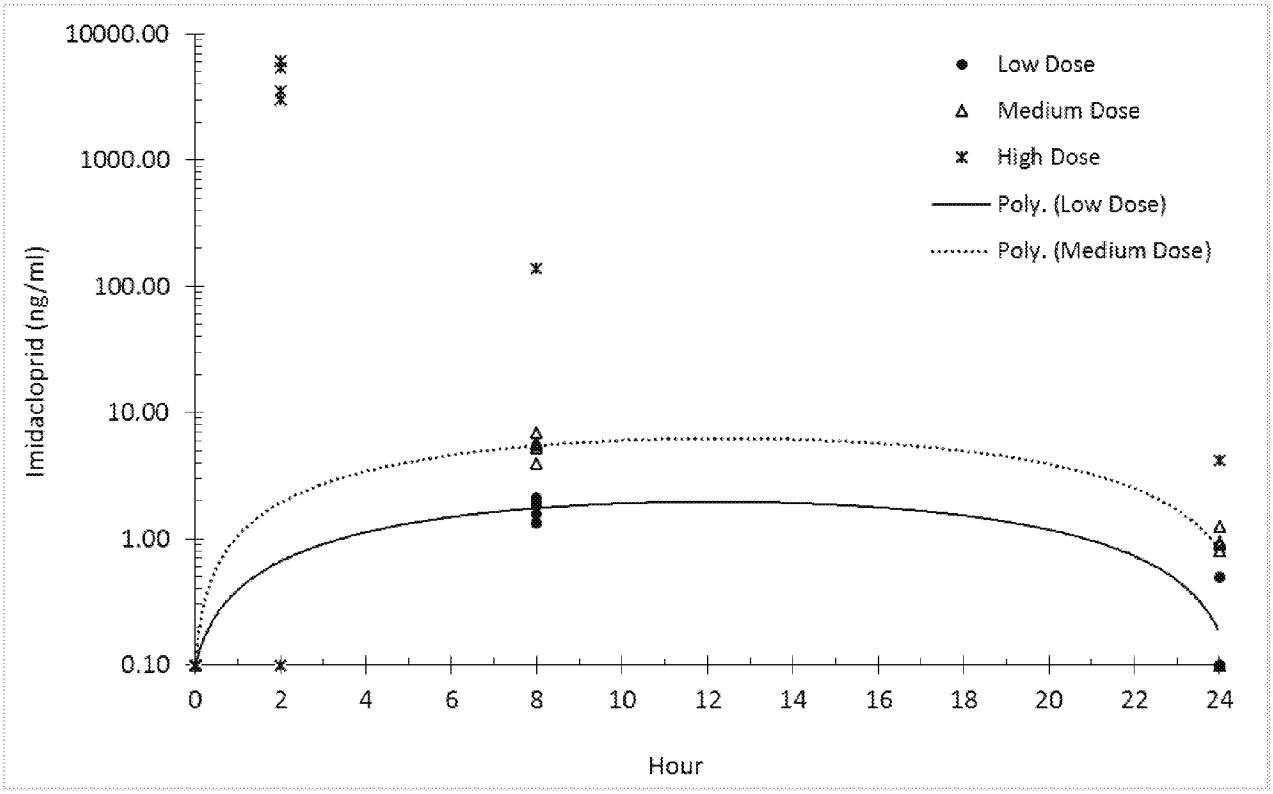


Figure 1. Changes in imidacloprid (IMI) concentrations in blood of dosed domestic chickens over time after one dose at University of Minnesota – College of Veterinary Medicine in 2015. IMI doses were 1%, 5%, and 20% of a reported IMI $LD_{50}$ for chickens (i.e., low, medium, and high dose groups, respectively). IMI detection limit is 0.10 or -1.0 $log_{10}$ ng/ml in blood. Data points overlap when plotted on x-axis minimum value. A polynomial (Poly) trend line was fit for the low- and medium-dosed birds, but could not be fit to the data from high-dosed birds because chickens in this dose group were euthanized within 24 hours due to animal welfare concerns. Thus, the high dose group is not directly comparable to the other dose groups.

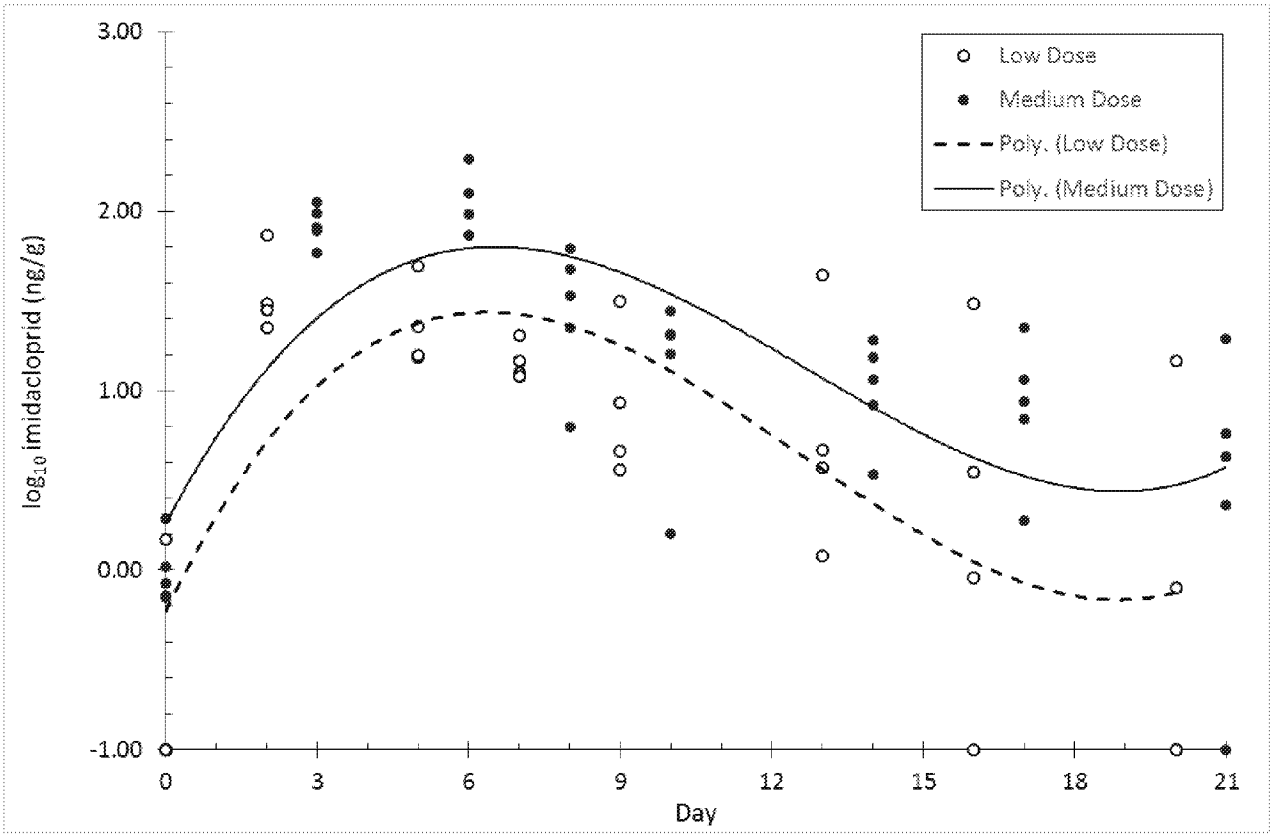


Figure 2. Changes in imidacloprid (IMI) concentrations in feces of dosed domestic chickens over time at University of Minnesota – College of Veterinary Medicine in 2015. Samples collected on day 0 were baseline samples, prior to exposure. Birds received a daily IMI dose for 7 days of 1% (low dose) and 5% (medium dose) of a reported IMI $LD_{50}$ for chickens. The last day of dosing occurred on day 7 of the 21 day experiment. IMI detection limit was 0.10 or -1.0 $log_{10}$ ng/g in feces. The high dose group is not included because samples were collected only on day 0 so no temporal trends could be determined. Chickens in the high dose group were euthanized within 24 hrs after dosing due to animal welfare concerns. Thus, the high dose group is not directly comparable to the other dose groups. Polynomial (Poly) trend lines were fit to the data for the low and medium dose groups.

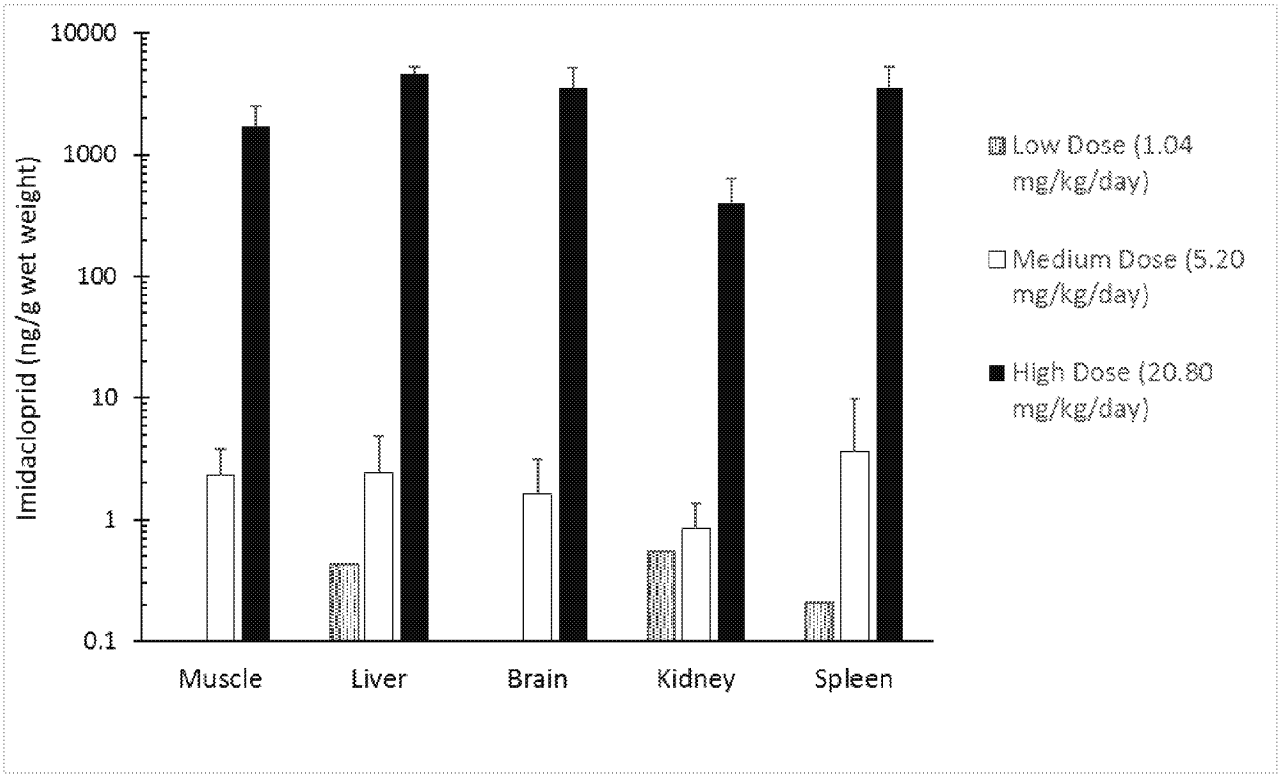


Figure 3. Concentrations of imidacloprid (geometric mean + SD ng/g wet tissue weight) in tissues of laboratory-exposed domestic chickens on experimental day 1 (high dose) or 21 (low and medium dose) at University of Minnesota – College of Veterinary Medicine in 2015. Data at the detection limit of 0.10 ng/g are not visible. Error bars represent the standard deviation of observations for a given group.