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

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SUMMARY OF FINDINGS

Neonicotinoid pesticides [e.g., imidacloprid (IMI), thiamethoxam (TMX), thiacloprid (THIA), clothianidin (CLO)] 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). Using several lines of evidence, we examined sub-lethal exposure and the potential for exposure of wildlife to these pesticides in agricultural landscapes of Minnesota in 2016 and 2017. We documented exposed seeds at the soil surface in plots at 35% of 71 fields sampled after planting. We also quantified the rate of seed spills during planting season and documented 329 seed spills in the 76 townships surveyed in the spring. We documented birds and mammals eating treated seeds through field studies with trail cameras. We quantified consumption of treated seeds for 11 species of birds and 9 species of mammals, and in many cases we estimated that more than 25% of the LD_{50}—the amount of ingested substance to kill 50% of a test sample—was ingested. Seed exposure experiments conducted under environmental conditions indicated that neonicotinoids are persistent on the seed surface for as long as 30 days in the environment, so wildlife can ingest neonicotinoids on treated seeds for at least 30 days after planting.

We also conducted laboratory experiments using domestic chickens (*Gallus gallus domesticus*) to identify non-lethal and lethal sampling methods that could lead to measurement of individual- and population-level exposure, including residues in the excreta and blood of birds. Mean residue concentrations in chickens dosed in the lab were highest in the brain. In decreasing order of concentration, residues were also detected in liver, spleen, muscle, blood, kidney, then feces. Residues in chicken fecal samples collected in the lab had the highest frequency of detection in all tissues tested.

Finally, we collected field samples from prairie grouse leks and from hunter-harvested birds to evaluate whether wild birds were exposed to sub-lethal doses. Seventy-three of 82 (89%) liver samples collected from sharp-tailed grouse (*Tympanuchus phasianellus*) and 32 of 45 (71%) greater prairie-chickens (*Tympanuchus cupido*) contained concentrations above the

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Method Limit of Quantification (MLOQ) for at least one neonicotinoid. Similarly, 95 of 109 (87%) sharp-tailed grouse fecal pellets and 51 of 59 (86%) fresh greater prairie-chicken fecal pellets collected from leks have been analyzed and had concentrations above the MLOQ for ≥1 neonicotinoid. Most of the detected concentrations were <10 ng/g, which explains why earlier studies with higher detection thresholds than the current study concluded a more rapid clearance of neonicotinoids from vertebrates than we found. Only 3 greater prairie-chicken livers and 9 sharp-tailed grouse livers had CLO concentrations >10 ng/g, and 3 greater prairie-chicken and 7 sharp-tailed grouse livers had IMI >10 ng/g. Similarly, only 2 greater prairie-chicken pellets and 5 sharp-tailed grouse pellets had CLO >10 ng/g, and 9 greater prairie-chicken and 14 sharp-tailed grouse pellets had IMI >10 ng/g. These results show that wildlife were exposed to neonicotinoids through treated seeds, a large proportion of prairie grouse in Minnesota had quantifiable residues of neonicotinoids, and wildlife may have experienced both sub-lethal and lethal effects. Further research is necessary to evaluate individual- and population-level effects of these rates of ingestion of neonicotinoid-treated seeds.

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 relatively 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) recently conducted a special registration review of neonicotinoid pesticides with an emphasis on pollinators (MDA 2016). 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 genetic and physiological factors including size, metabolic, 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 than lethal exposure if mortality events are relatively small and carcasses rapidly removed by scavengers. Sub-lethal effects in birds in the lab include hyporeactivity, lack of coordination, wing drop, immobility, disruption of migratory coordination, eggshell thinning, reduced egg hatching rate, impaired testicular function, and low weight in chicks (Cox 2001, Lopez-Antia et al. 2013 and 2015, Tokumoto et al. 2013, Mineau and Palmer 2013, Eng et al. 2017). Avian reproduction can be affected by consumption of just 1/10th 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 red-legged partridge (*Alectoris rufa*) 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). Use of neonicotinoid "treated articles," such as seed, is not currently tracked by the U.S. government due to the exemption in 40CFR §152.25(a). Yet, almost all corn planted in the Midwestern U.S. has been treated with these pesticides (Stokstad 2013); most soybean, wheat, and sunflower seeds are treated also; and neonicotinoids 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 generally 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." Despite these calls for field studies and the time elapsed, the information deficits identified have still not been sufficiently addressed. Importantly, the U.S. still lags behind Europe (Berny et al. 1999, Lopez-Antia et al. 2013, 2016) in field-based studies focused on neonicotinoids and wildlife. We are therefore conducting a study to determine whether wild birds are exposed to neonicotinoid-treated seeds in agricultural landscapes in Minnesota. Preliminary data from our ongoing studies are reviewed below.

OBJECTIVES

The overarching objective of our research was to ascertain whether birds are exposed to neonicotinoid-treated seeds in agricultural landscapes. Specifically, we aimed to:

1- Quantify the rate of seed spillage and surface seed exposure after planting within fields.
2- Identify birds consuming neonicotinoid-treated seeds and quantify consumption per foraging bout.
3- Quantitatively link exposure and chemical residues in tissue, blood, and 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.

STUDY AREA

We conducted the field portions of our study in agricultural regions of Minnesota. Most field components were conducted in the agriculturally-dominated western portion of the state including the quantification of actual seed spills (Figure 1a), seeds on the soil surface and seed consumption at simulated seed spills (Figure 1b) in the spring. Field samples of prairie grouse came from the northwestern part of this region and also the east-central part of the state where agriculture was present but comprised a smaller proportion of the landscape (Figure 1c).

METHODS

Quantifying Seed Spills

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 actual 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 and overfilling hoppers is similar for fields that are 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 ≥50 miles of roads and ≥50% of the area in corn, soybeans, and/or wheat production using the DOT Roads Layer (DOT 2008) and 2014 Cropland Data Layer (USDA-NASS 2015), respectively, in ArcGIS. These criteria were used to select townships with visual access to fields from roads while also not being 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. We surveyed a total of 76 townships 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 of a quarter-section (i.e., 40 acres). We recorded each quarter of a quarter-section in agricultural production, whether any part of it was recently planted (i.e., before 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 contained neonicotinoid-treated seed, we collected seeds from accessible spills (e.g., along public roads and rights-of-way) and quantified 7 neonicotinoids (Chen et al. 2014).

Quantifying Seeds on the Soil Surface

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 (i.e., 1 plot at vertex, 2 plots at 15 m, and 2 plots at 30 m). This approach incorporated sampling parallel and perpendicular to planting rows, and we suspected that seed exposure would be greater at the end of rows where planters turn sharply 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.

In 2016, we sampled 36 fields on DNR-managed Wildlife Management Areas (WMAs) that were farmed by private individuals under contract through Cooperative Farming Agreements (CFAs), 2 privately farmed fields on private land where we had permission, and 10 fields farmed by DNR staff on WMAs. In 2017, we sampled 6 privately farmed fields in CFAs and 17 privately owned and farmed fields with landowner consent. During 2017, neonicotinoid-treated seed was not permitted on WMAs. When seeds were exposed, we could determine whether they were treated; however, we did not dig up seeds for confirmation. In 4 cases, 2 fields were known to be planted by the same farmer, but in 3 cases, the fields were planted to different crop types, with different planting equipment used for each crop type in 2 of 3 cases where equipment type used was known.

Quantifying Decay of Neonicotinoids on Treated Seeds on the Soil Surface

To determine how long neonicotinoids persist on the seeds left on the soil surface we distributed hundreds of seeds on the soil surface of a tilled field near Bemidji to experience UV, microbial, rainfall, and other ambient conditions. After environmental exposure for 0, 1, 2, 4, 8, 16, and 30 days, we collected 5-7 seeds of each type to quantify decay of neonicotinoids under environmental conditions. We recorded daily precipitation and cloud cover during the experiment. We conducted the experiment in 2016 with 2 types of commercially available corn seed treatments (CLO and TMX) and commercially treated soybeans (IMI). In 2017, we repeated the experiment, but also put out wheat seeds (CLO, but the seed treatment was applied locally rather than through an industrial application). After field collection, seeds were stored frozen until shipping to a laboratory at Southern Illinois University Carbondale (SIUC) for neonicotinoid analysis.

Documenting Consumption of Treated Seeds

In 2016, we selected 12 WMAs to place trail cameras to observe wildlife consuming seeds at simulated spills in planted fields. 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). 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 DNR managers reported difficulty finding seeds that had not been treated. We prioritized this portion of the study in 2016 because farmers and managers were prohibited from planting neonicotinoid-treated seeds on WMAs beginning in 2017.

Camera locations were selected to minimize risk of theft and to view a recently planted field to document foraging at a simulated seed spill and on exposed or submerged seeds or seedlings. In 2016, spills were simulated with 1000 corn (n = 15 spills) or soybean seeds (n = 2 spills) to allow determination of the time it takes for birds to discover spills and the number of seeds consumed in each foraging bout by individual animals. Additionally, we placed cameras at 2 fields on privately-owned land where we had obtained permission. Cameras were deployed in each location for 3–6 weeks after planting. At each field, 2 motion-activated cameras were deployed—one that captured 1 image/sec in still photos and one that captured 1 min of video when triggered by motion. The camera set for still photos also took photos 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 were examined to identify species of wildlife consuming seeds and the number of seeds consumed per foraging bout.

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. We simulated 20 more corn spills, 23 soybean spills, and 9 wheat spills of 1000 seeds each. 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. Cameras were programmed to capture a 1 min video whenever the motion sensor was triggered. We checked cameras once weekly to replace batteries and data cards and deployed cameras in each location for 2–3 weeks. When we checked simulated spills, we restocked with an additional 1000 seeds of the same seed type if 25-50% of the seeds remained but switched to a different seed type (after removing any remaining seeds) if <25% remained.

Linking Field and Laboratory Exposure Concentrations in Birds

We quantitatively linked field sample concentrations to laboratory exposure concentrations through work with University of Minnesota-College of Veterinary Medicine (UMN-CVM) and SIUC. We determined how many days post-exposure that imidacloprid (i.e., the most common seed treatment in Minnesota, J. Zachmann, MDA, pers. comm.) was detectable in both non-lethally and lethally collected samples from dosed birds. 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 chickens (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, 5.20 mg/kg/day, and 20.80 mg/kg/day (i.e., low, medium, and high dosage, respectively). The LD_{50} is the single dose that is expected to be lethal to 50% of test subjects.

The LD_{50} would be reached if chickens ingested ~260–946 corn seeds (depending on application rate to seeds, which varies among seed companies). Stated differently, 3–10 seeds is comparable to the low, or 1%, LD_{50} dose. Thus, these were realistic doses. Prairie grouse (0.6–1.2 kg) are smaller than chickens and thus smaller doses (e.g., 104–780 seeds for the low-dose treatment, depending on bird weight) would be expected to produce similar results. Other neonicotinoids have a higher LD_{50} than IMI, so lethality would be expected at much higher seed ingestion levels for those pesticides.

The full laboratory experiment was completed only for chickens in the low- and medium-dosage groups because chickens in the high-dosage 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. Chickens that were considered at endpoint and euthanized had blood samples taken immediately before euthanasia. The low-dosage group was sampled for feces 1 day earlier than the medium group due to logistical challenges. Samples of internal organs (i.e., brain, kidney, liver, spleen) and muscle were taken from chickens that died during the treatment period or on day 21, whichever came first. Chickens 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 were performed to gain a preliminary sense of how IMI concentrations changed over time and in response to dose on a tissue-specific basis. According to best practices, we 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 we obtain the full dataset, including metabolites (i.e., neonicotinoids modified through metabolic processes) and feed concentrations.

Detecting Neonicotinoids in Free-ranging Birds

We also collected samples from wild birds using both invasive and non-invasive methods 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 and again in 2017 for this study. Samples were stored frozen until shipped to the lab at SIUC. Hunters also voluntarily submitted harvested prairie grouse in fall 2015, 2016, and 2017. Tissues and fecal pellets are being tested for thiacloprid (THIA), acetamiprid (ACE), thiamethoxam (TMX), IMI, clothianidin (CLO), dinotefuran (DIN) and nitenpyram (NTP).

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

RESULTS

Quantifying Seed Spills

We observed 212 large seed spills that were visible from the road during surveys in 2016 and 117 spills during surveys in 2017. However, we missed the peak of planting in many

of the townships surveyed because both the springs of 2016 and 2017 were very wet and crops were planted later than usual. Planting in 2017 was later than in 2016, and we observed standing water in many fields during the spring planting season. At the time of our road-based surveys in 2016, 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, amounting to 59% of the acres surveyed having been planted at the time of our survey. 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. In 2017, 40,110 acres of corn, 23,556 acres of soybeans, and 33,749 acres of wheat, and 14,957 acres of other crops were planted during our surveys, or 23% of acres surveyed were planted at the time of our survey. Spill rates of 2 spills/10,000 ac corn, 25 spills/10,000 ac soybean, 6 spills/10,000 ac wheat planted were calculated. 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 (NASS); last accessed 5 June 2017 National Agricultural Statistics Service], we estimate nearly 15,000 large seed spills statewide in 2016 and expect that if there is a bias, our estimates are biased low. In 2017, 8,050,000 ac of corn, 8,150,000 acres of soybeans, and 1,160,000 acres of spring wheat were planted (NASS; last accessed 5 March 2018 National Agriculture Statistics Service), which extrapolates to ~22,000 spills during the planting season. Spills increased as we moved from south to north, and the proportion of fields planted during our surveys also increased as we moved south to north.

Quantifying Seeds on the Soil Surface

We documented exposed seeds at the soil surface in plots in 25 of the 71 fields where we sampled 10 1-m^2 plots in 2016 and 2017, and when areas outside plots were included, 40 fields had exposed seeds at the soil surface (Table 3). Seeds were exposed in $\geq$1 centrally located plot in 20% of fields measured. Exposed seeds were detected in $\geq$1 corner plot of 30% of fields measured. The quantity of exposed seeds on the surface of fields was 0.47 seeds/m^2 (range: 0-69) in the center of fields and 0.77 seeds/m^2 (range: 0-51) in the edges of fields, which is an order of magnitude lower than that reported by Lopez-Antia et al (2016). Most (72%) of the fields we measured were planted to corn, 24% were planted to soybeans, and 4.2% were planted to wheat (Table 4). Most (73%) sampled fields were on public land but 81% 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 the seed type.

Quantifying Decay of Neonicotinoids on Treated Seeds on the Soil Surface

Neonicotinoids decayed on the surface of seeds relatively quickly, but concentrations exceeding 10 ng/g were present on all seeds after 16 days, and on IMI treated seeds after 30 days (Figure 2). We did not have a 30 day sample for CLO treated seeds because no seeds remained on the soil surface after 30 days, presumably due to wildlife consumption because the seeds were not removed from the tilled field by people.

Documenting Consumption of Treated Seeds

We reviewed images collected by trail cameras at simulated spills during spring 2016 (*n* = 188,399 photos and 12,602 videos) and 2017 (n = 39,653 videos). We documented ring-necked pheasants (*Phasianus colchicus*), Canada geese (*Branta canadensis*), American crows (*Corvus brachyrhynchos*), mourning doves (*Zenaida macroura*), wild turkeys (*Meleagris gallapavo*), blue jays (*Cyanocitta cristata*), brown thrasher (*Toxostoma rufum*), rose-breasted grosbeak (*Pheucticus ludovicianus*), various species of sparrows (Emberizidae) and blackbirds (Icteridae), as well as white-tailed deer (*Odocoileus virginianus*), black bears (*Ursus americanus*), raccoons (*Procyon lotor*), rodents, Eastern cottontails (*Sylvilagus floridanus*) and white-tailed jackrabbits (*Lepus townsendii*) consuming treated seeds. Consumption rates (seeds/min), the number of seeds eaten per 1 min video, and the total seeds eaten by an individual in consecutive videos are indicated in Table 1.

To estimate the toxicity of consuming neonicotinoid treated seeds, we estimated species-specific LD_{50} concentrations using standard metabolic scaling procedures (EPA T-REX[3]) with estimated toxicity values for surrogate species, the mass of surrogate species, and product-labeled concentrations of chemical on a treated seed (in mg/seed; Bayer Crop Science and Syngenta). Toxicity values (LD_{50} in mg/kg-bw) for surrogate species were acquired from EPA draft risk assessments or other documents (DeCant and Barrett 2010, Anon 2012, EPA_HQ-OPP-2011-0865-0242, EPA-HQ-OPP-2008-0844-1256, EPA-HQ-OPP-2011-0581-0093) to create the potential toxicity assessment (Table 2) for species observed consuming treated seeds in images. These metrics are useful for the assessment of risk in birds and mammals. In summary, potential exposure concentrations were much closer to estimated LD_{50} concentrations for birds than mammals.

Linking Field and Laboratory Exposure Concentrations in Birds

We collected 72 blood samples; 100 fecal samples; 15 samples of muscle, brain, liver, and kidney; and 103 eggs during laboratory IMI exposures of chickens. Based on a detection limit of 0.10 ng/g, 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; Table 5). Blood concentrations increased from the first samples taken at the start of the experiment (hr 0) to hr 8 and declined again at hr 24 (Figure 3); after this time, samples did not contain detectable IMI except for one 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 4). As expected, the low dose group tended to exhibit lower IMI fecal concentrations than birds in the medium dose group. 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

[3] EPA T-REX guide

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 3) and thus may be more applicable to field applications where time from chemical exposure will be more variable.

IMI was measured in internal organs which were collected on the final day of the experiment, depending on when birds were euthanized (Figure 5). 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 6).

For analytical method quality assurance and control, we used matrix spiked recovery tests, procedural blanks, and recoveries of surrogate standards. IMI (25 ng) was spiked into muscle (n = 5) or blood (n = 5) and analyzed. Mean (± SD) recoveries were 86.7 ± 5.8% and 90.9 ± 4.9% in tissue or blood, respectively. One procedural blank was processed for every 10 samples, and no target compound was detected in any blanks. Good analytical performance was indicated by surrogate standards with recoveries ranging from 75% to 98%. Similar methods were used for THIA, ACE, TMX, and CLO and the method limit of quantification was calculated by multiplying the standard deviation from replicates with a Student's t-value appropriate for a 99% confidence level. Thus, the method limit of quantification (MLOQ) for IMI was 0.3 ng/g in tissue and 0.4 ng/mL in blood, for THIA was 0.7 ng/g and 0.6 ng/mL, for ACE was 0.7 ng/g and 0.8 ng/mL, for TMX 0.8 ng/g and 0.8 ng/mL, and for CLO was 0.7 ng/g and 0.7 ng/mL in tissue and blood respectively, Minimum detectable concentrations were lower and ranged 0.1–0.3 ng/g for the 5 neonicotinoids, but we took a more conservative approach for reporting and interpretation.

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 (no sample collection occurred in 2016). 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, 17 prairie-chickens, 33 sharp-tailed grouse, and 2 pheasants during fall 2016, and 17 prairie-chickens and 27 sharp-tailed grouse during fall 2017.

Seventy-three of 82 (89%) livers collected from hunter-harvested sharp-tailed grouse, 32 of 45 (71%) greater prairie-chicken livers, and 3 of 3 sharptail/prairie-chicken hybrids from hunter-submitted samples had concentrations above the MLOQ for at least one neonicotinoid. Three of 3 blood samples analyzed tested negative for neonicotinoids. Dinotefuran and NTP were not detected in any samples. Neonicotinoids above the MLOQ in prairie-chicken livers included IMI (64%), CLO (27%), and THIA (2%) and in sharp-tailed grouse livers included IMI (79%), CLO (37%), THIA (5%), and ACE (1%). Maximum concentrations of neonicotinoids in prairie-chicken livers were 22.0 ng/g IMI, 15.0 ng/g CLO, and 1.1 ng/g THIA. (Note that ACE and TMX were reported in a previous report, but detected concentrations were below the

MLOQ; 0.21 ng/g, ACE, and 0.43 ng/g TMX). Maximum concentrations detected in livers of harvested sharp-tailed grouse were 84.5 ng/g IMI, 21.0 ng/g CLO, 1.18 ng/g THIA, 0.71 ng/g ACE, and 0.5 ng/g TMX, again with TMX below the more conservative MLOQ. Similarly, 51 of 59 (86%) fresh prairie-chicken fecal pellets and 95 of 109 (87%) sharp-tailed grouse pellets collected from leks during springs of 2015 and 2017 contained concentrations above the MLOQ for at least one neonicotinoid. The most commonly detected neonicotinoid in the greater prairie-chicken fecal pellets was IMI (51%), followed by CLO (37%), and THIA (3%). 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 14.0 ng/g, 44.8 ng/g, 1.05 ng/g, respectively. In sharp-tailed grouse pellets, neonicotinoids above the MLOQ were IMI (62%), CLO (40%), and THIA (4%). Maximum concentrations were 39.7 ng/g IMI, 32.3 ng/g CLO, 0.9 ng/g THIA, with ACE and TMX below the MLOQ (0.2 ng/g and 0.5 ng/g, respectively). However, most of the detected concentrations were <10ng/g, which is below the detection limit in tissues in some other laboratories. Only 3 greater prairie-chicken livers and 9 sharp-tailed grouse livers had CLO concentrations >10ng/g, and 3 greater prairie-chicken and 7 sharp-tailed grouse livers had IMI>10ng/g. Similarly, only 2 greater prairie-chicken pellets and 5 sharp-tailed grouse pellets had CLO >10ng/g, and 9 greater prairie-chicken and 14 sharp-tailed grouse pellets had IMI >10ng/g.

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, indicating that this was either their first exposure or that previous exposures were not recent enough to detect.

DISCUSSION

We found that neonicotinoid-treated seed is common on the landscape during the spring planting season, both on seeds available on the soil surface and in seed spills. We also documented numerous avian and mammalian species consuming treated seeds at simulated spills, some of which ingested amounts that would be expected to produce lethal and sub-lethal effects. Samples obtained from wild birds during the fall hunting season also indicated recent exposure in a large proportion of harvested birds, which is consistent with consumption of treated seeds during planting of winter wheat in September and October in Minnesota. Indeed, several of the hunter-submitted sharp-tailed grouse carcasses contained wheat. These findings indicate a need for much more study into the exposure rates of wildlife to neonicotinoids. Population-level effects are possible based on the consumption rates, availability of treated seed, and persistence of neonicotinoids on seeds under environmental conditions that we observed. Thus, lethal and sub-lethal effects should receive more attention in wild populations, especially in granivorous species that consume seeds as part of their diet.

Field studies on neonicotinoids in vertebrates have been infrequent to date, in part due to methodological obstacles for field detection and in part due to the difficulty of isolating variables in field settings where variables cannot be easily controlled. We identified several

methodological options that can be applied in field situations, including detection of residues in feces and tissues. Notably, fecal samples provide a non-invasive means to detect exposure in birds, which can be especially important for species of concern. Fecal samples also could be collected from the GI tract of live birds or from hunter-killed birds. For game species and more common species, internal organs like livers can also serve as an indicator of neonicotinoid exposure in lethal collections and livers are fairly easy for non-specialists to locate. 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*). We had few ingesta samples, but our results also indicated that liver and kidney provide more consistent imidacloprid concentrations than other tissues.

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. Refining non-invasive collection is necessary because UV light can and microbial degradation may degrade neonicotinoids (Lu et al. 2015; Lu et al. 2016; Ma et al. 2014). Thus pellet freshness is an important consideration. 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). Other studies have had 10-fold lower detection thresholds in tissues, which explains the discrepancy between our study and others.

The highest concentration of IMI detected in livers of harvested prairie grouse (84.5 ng/g) 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 IMI detected in field-collected feces (39.7 ng/g) was consistent with the 1% dose group, lower than the 5% dose groups within 3 days of exposure, and was generally higher than both dose groups 2 weeks post-exposure, although samples varied substantially. We cannot know if this indicates a higher initial exposure or how much the passage of time since exposure might have reduced these levels, but given that 1% LD_{50} (1.04 mg/kg) is comparable to the dose received after consuming 3–10 corn seeds and that IMI can be detected in tissues for as long as 21 days post-exposure, we consider it likely that this finding reflects a high initial exposure to IMI.

This research provides evidence contrary to several popularly held beliefs that wildlife do not eat treated seeds because they are unpalatable, that seeds are always drilled below the soil surface and are thus not available for wildlife, and that packaging labels are sufficient to protect wildlife from harmful effects. We encourage other researchers to replicate our study, and to pursue additional field studies of wildlife, to ensure that objective data are available to evaluate the risks of neonicotinoids to wildlife.

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Table 1. Birds and mammals documented eating seeds at simulated spills by seed type (corn, soybean, & wheat in separate sections of the table). Consumption rates (seeds consumed/min), the range of seeds consumed in 1 min videos, and the maximum amount of seeds consumed by an individual in consecutive videos.

Species	Scientific name	Corn Consumption Rate (seeds/min)	Sample size	Range (seeds eaten per 60 s video)	Max seeds eaten per feeding bout
Common grackle	*Quiscalus quiscula*	3.2	27	1-5	5
Blue jay	*Cyanocitta cristata*	27.7	4	2-4	6
Ring-necked pheasant	*Phasianus colchicus*	15.3	9	1-21	21
Red-winged blackbird	*Agelaius phoeniceus*	1.9	28	1-6	6
Brown thrasher	*Toxostoma rufum*	2.46	5	1-3	3
American crow	*Corvus brachyrhynchos*	28.1	16	1-24	24
Black-billed magpie	*Pica hudsonia*	12	1	2	2
Wild turkey	*Melagris gallapavo*	174.2	2	1-150	150
White-tailed deer	*Odocoileus virginianus*	54.2	8	5-111	650
13-lined ground squirrel	*Ictidomys tridecemlineatus*	7.7	24	1-13	22
Raccoon	*Procyon lotor*	11.9	32	4-21	268
Eastern cottontail	*Sylvilagus floridanus*	3.1	14	1-6	35
White-tailed jackrabbit	*Lepus townsendii*	3.8	5	3-5	43
Eastern gray squirrel	*Sciurus carolinensis*	3.1	4	1-4	23
Fox squirrel	*Sciurus niger*	3.3	9	2-6	48
Striped skunk	*Mephitis mephitis*	13	1	13	13
Red fox kit	*Vulpes vulpes*	2.1	5	1-3	3
Red fox adult	*Vulpes vulpes*	n/a	2	1-2	2

Species	Scientific name	Soybean Consumption Rate (seeds/min)	Sample size	Range (seeds eaten per 60 s video)	Max seeds eaten per feeding bout
Ring-necked pheasant	*Phasianus colchicus*	18.9	21	1-36	68
Canada goose gosling	*Branta canadensis*	33.6	2	3-7	9
White-tailed deer	*Odocoileus virginianus*	107.6	36	3-317	800
13 lined ground squirrel	*Ictidomys tridecemlineatus*	6.9	15	1-14	14
Raccoon	*Procyon lotor*	9.7	4	5-8	61
Eastern cottontail	*Sylvilagus floridanus*	9.4	12	1-14	14
Fox squirrel	*Sciurus niger*	1.0	1	1	1

Species	Scientific name	Wheat Consumption Rate (seeds/min)	Sample size	Range (seeds eaten per 60 s video)	Max seeds eaten per feeding bout
Red-winged blackbird	*Agelaius phoeniceus*	10.5	2	2-5	5
American crow	*Corvus brachyrhynchos*	29.8	4	4-30	61
Mourning dove	*Zenaida macroura*	16.2	32	1-31	73
Song sparrow	*Melospiza melodia*	1.6	6	1-2	2
Wild turkey	*Meleagris gallapavo*	199.7	5	153-215	700

Table 2. Estimation of potential avian and mammalian acute toxicity from different levels of treated seed consumption for focal species using surrogate species and metabolic scaling approaches as described in EPA's T-REX model. Mammalian scaling factor was 0.75 and avian scaling factor was 1.15. Neonicotinoid chemicals (CHEM) evaluated were clothianidin (CLO), imidacloprid (IMI), and thiamethoxam (TMX).

CHEM	Focal species	Seed	Surrogate	Surrogate LD_{50} (mg/kg)	Estimated LD_{50} (mg/kg)	Max % of LD_{50}	Seeds (#) for LD_{50}	Time to LD_{50} (mins)
CLO	American crow	Corn	Bobwhite quail *Colinus virginianus*	200	174	38.2	63	2
CLO	Black-billed magpie	Corn	Bobwhite quail	200	200	7.0	29	2
CLO	Blue jay	Corn	Bobwhite quail	200	224	39.4	15	0.5
CLO	Brown thrasher	Corn	Bobwhite quail	200	228	21.9	14	6
CLO	Common grackle	Corn	Bobwhite quail	200	216	26.8	19	6
CLO	Red-winged blackbird	Corn	Bobwhite quail	200	239	56.6	11	6
CLO	Ring-necked pheasant	Corn	Japanese quail *Coturnix japonica*	423	271	5.5	379	25
CLO	Wild turkey	Corn	Japanese quail	423	221	12.5	1195	7
CLO	American crow	Wheat	Bobwhite quail	200	174	1.8	3384	114
CLO	Mourning dove	Wheat	Bobwhite quail	200	206	5.6	1300	80
CLO	Red-winged blackbird	Wheat	Bobwhite quail	200	239	0.9	571	54
CLO	Song sparrow	Wheat	Bobwhite quail	200	259	0.6	363	227

CHEM	Focal species	Seed	Surrogate	Surrogate LD_{50} (mg/kg)	Estimated LD_{50} (mg/kg)	Max % of LD_{50}	Seeds (#) for LD_{50}	Time to LD_{50} (mins)
CLO	Wild turkey	Wheat	Japanese quail	423	221	1.1	64457	323
IMI	Blue jay	Corn	House sparrow *Passer domesticus*	41	34	280	2	0.1
IMI	Common grackle	Corn	House sparrow	41	34	183	3	0.9
IMI	Red-winged blackbird	Corn	House sparrow	41	37	387	2	0.8
IMI	Ring-necked pheasant	Soy	Japanese quail	17	11	83.5	81	4
TMX	Blue jay	Corn	Mallard *Anas platyrhynchos*	576	804	11.0	55	2
TMX	Common grackle	Corn	Mallard	576	804	7.2	70	22
TMX	Red-winged blackbird	Corn	Mallard	576	889	15.2	40	21
IMI	White-tailed deer	Corn	Mouse *Mus musculus*	131	1063	1.0	65471	1208
IMI	13-lined ground squirrel	Corn	Mouse	131	233	66.6	33	4
IMI	Raccoon	Corn	Mouse	131	700	3.3	8098	681
IMI	Eastern cottontail	Corn	Mouse	131	384	8.7	401	129
IMI	White-tailed jackrabbit	Corn	Mouse	131	479	3.5	1216	320
IMI	Eastern gray squirrel	Corn	Mouse	131	297	20.8	111	36
IMI	Fox squirrel	Corn	Mouse	131	328	26.1	184	56
IMI	Striped skunk	Corn	Mouse	131	470	1.2	1105	85
IMI	Red fox adult	Corn	Mouse	131	595	0.1	3598	1799
IMI	White-tailed deer	Soy	Mouse	131	1063	0.2	374916	3484
IMI	13-lined ground squirrel	Soy	Mouse	131	233	7.4	189	27
IMI	Raccoon	Soy	Mouse	131	700	0.1	46375	4781
IMI	Eastern cottontail	Soy	Mouse	131	384	0.6	2296	244
IMI	Fox squirrel	Soy	Mouse	131	328	0.1	1052	1052

CHEM	Focal species	Seed	Surrogate	Surrogate LD_{50} (mg/kg)	Estimated LD_{50} (mg/kg)	Max % of LD_{50}	Seeds (#) for LD_{50}	Time to LD_{50} (mins)
CLO	White-tailed deer	Corn	Mouse	427	3466	0.3	228769	4221
CLO	13-lined ground squirrel	Corn	Mouse	427	759	19.1	115	15
CLO	Raccoon	Corn	Mouse	427	2282	0.9	28297	2378
CLO	Eastern cottontail	Corn	Mouse	427	1251	2.5	1401	452
CLO	White-tailed jackrabbit	Corn	Mouse	427	1562	1.0	4248	1118
CLO	Eastern gray squirrel	Corn	Mouse	427	967	5.9	387	125
CLO	Fox squirrel	Corn	Mouse	427	1070	7.5	642	195
CLO	Striped skunk	Corn	Mouse	427	1532	0.3	3861	297
CLO	Red fox	Corn	Mouse	427	1940	0.0	12573	6287
TMX	White-tailed deer	Corn	Rat *Rattus norvegicus*	1563	7135	0.1	470899	8688
TMX	13-lined ground squirrel	Corn	Rat	1563	1563	9.3	238	31
TMX	Raccoon	Corn	Rat	1563	4697	0.5	58247	4895
TMX	Eastern cottontail	Corn	Rat	1563	2575	1.2	2884	930
TMX	White-tailed jackrabbit	Corn	Rat	1563	3215	0.5	8744	2301
TMX	Eastern gray squirrel	Corn	Rat	1563	1991	2.9	796	257
TMX	Fox squirrel	Corn	Rat	1563	2203	3.6	1322	401
TMX	Striped skunk	Corn	Rat	1563	3154	0.2	7948	611
TMX	Red fox adult	Corn	Rat	1563	3994	0.0	25880	12940
TMX	White-tailed deer	Soy	Rat	1563	7135	0.0	3893016	36180
TMX	13 lined ground squirrel	Soy	Rat	1563	1563	0.7	1964	285
TMX	Raccoon	Soy	Rat	1563	4697	0.0	481541	49643
TMX	Eastern cottontail	Soy	Rat	1563	2575	0.1	23844	2537
TMX	Fox squirrel	Soy	Rat	1563	2203	0.0	10928	10928

Table 3. Exposed seeds on the soil surface after planting in 3 categories of field types. Cooperative Farming Agreements (CFAs) are privately farmed areas on public land. Public fields were farmed by DNR staff with older planting equipment. Private lands were fields where we obtained landowner permission to survey fields after planting. We did not dig up seeds to determine whether they were treated, so if no seeds were on the surface, we did not know whether the seeds were treated.

Field type	# fields	Treated (T) or not treated (N)	Exposed seeds in center plots	Exposed seeds in corner plots	Exposed seeds outside plots	Spills
CFA (private equipment, public land)	42	18T, 2N	4 (10%)	4 (10%)	17 (40%)	4 (10%)
Public (old equipment, DNR staff)	10	3T, 4N	3 (30%)	5 (50%)	7 (70%)	0 (0%)
Private (pvt equipment)	19	13T, 4N	7 (37%)	12 (63%)	14 (74%)	8 (42%)
Total	71	34T, 10N	14 (20%)	21 (30%)	38 (54%)	12 (17%)

Table 4. Exposed seeds on the soil surface after planting by crop type. We did not dig up seeds to determine whether they were treated, so if no seeds were on the surface, seed treatment was unknown.

Field type	# fields	Exposed seeds in center plots	Exposed seeds in corner plots	Exposed seeds outside plots	Spills
Corn treated	24	2	4	21	5
Corn untreated	1	0	0	0	0
Corn unknown if treated	26	0	0	0	0
Total (and %) corn fields	51	2 (4%)	4 (8%)	21 (41%)	5 (10%)
Soybean treated	9	5	8	8	4
Soybean untreated	8	6	8	8	1
Soybean unknown if treated	0	0	0	0	0
Total (and %) soybean fields	17	11 (65%)	16 (94%)	16 (94%)	5 (29%)
Wheat treated	1	1	0	0	1
Wheat untreated	1	0	1	1	1
Wheat unknown if treated	1	0	0	0	0
Total wheat fields[a]	3	1	1	1	2
Total (and %) all field types	71	14 (20%)	21 (30%)	38 (54%)	12 (17%)

[a]Due to low numbers of sampled wheat fields, percentages are not provided.

Table 5. Summary of imidacloprid detections in domestic chicken blood and feces in each of 3 dose groups. 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 6. Summary of tissue concentrations of imidacloprid in all laboratory-exposed chickens for all dose groups combined.

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.

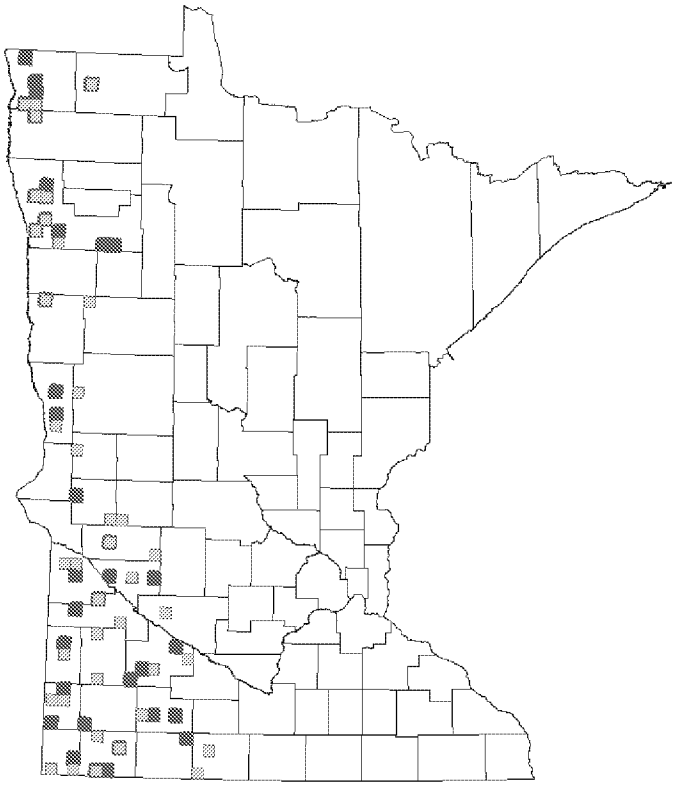

Figure 1a. Townships (*n* = 76) in Minnesota surveyed for seed spills during planting season in 2016 (dark gray), 2017 (light gray), and both years (light gray outlined with dark gray).

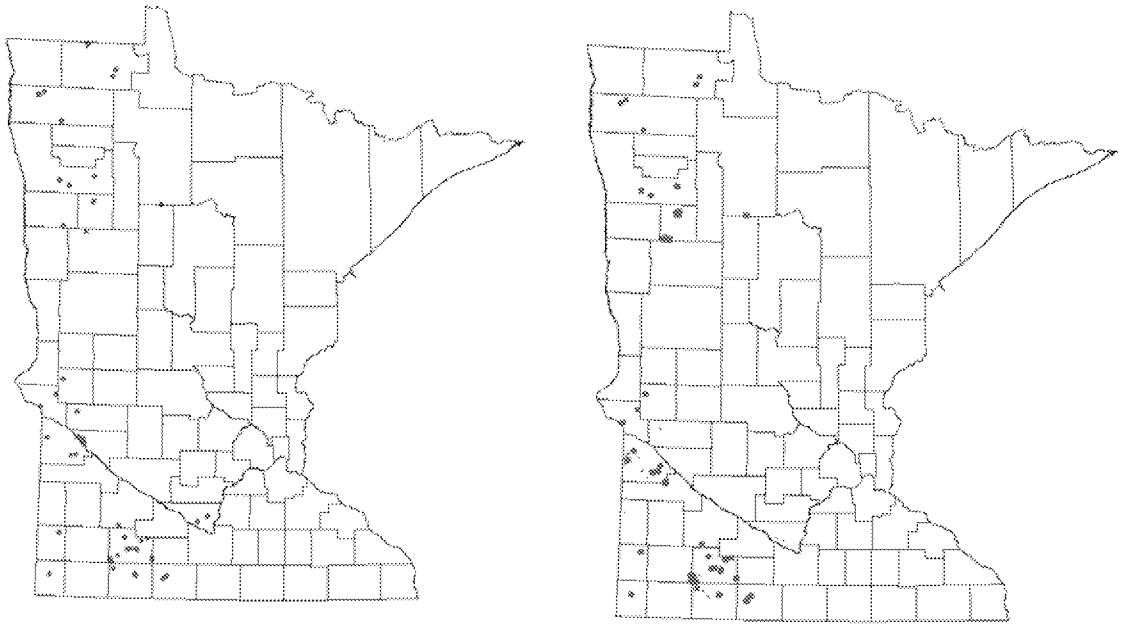

Figure 1b. Location of fields where seeds were measured on the soil surface after planting (left) and where cameras were placed at simulated spills (right). Fields are indicated as larger than their actual size to show their relative locations at a statewide scale; thus, some fields cannot be distinguished separately from other nearby fields (e.g., 17 fields on Lac Qui Parle Wildlife Management Area appear to be a single large site). Generally, the same sites were used, but some differences occurred related to the stage after planting during our visits and the ability to return to sites to remove cameras.

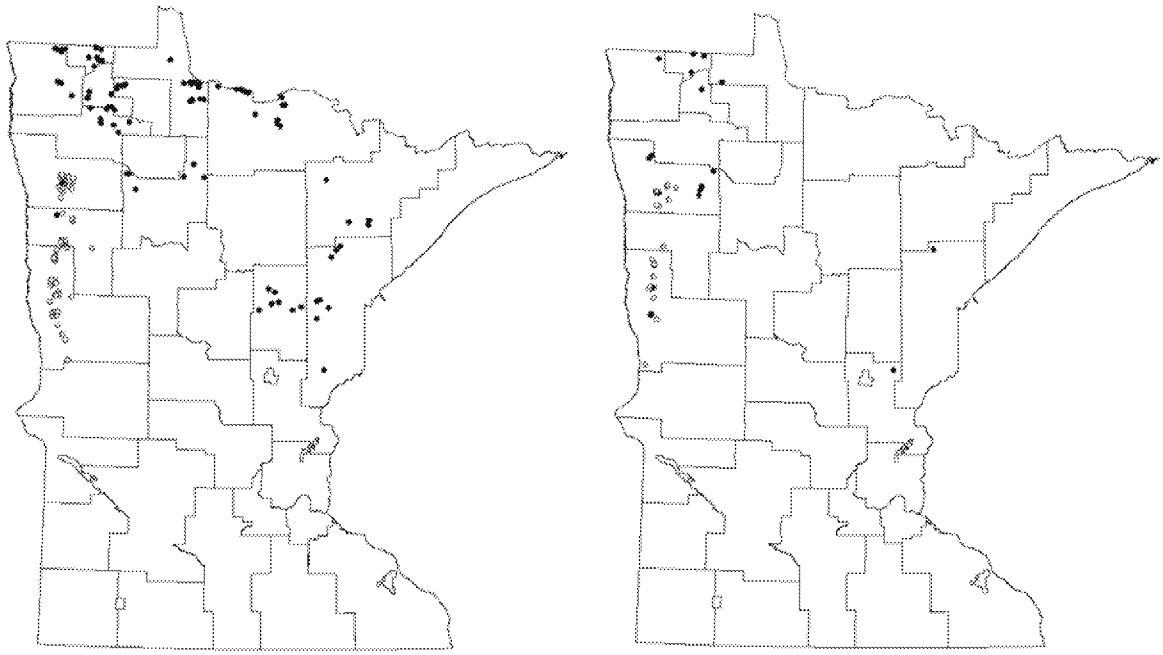

Figure 1c. Locations where sharp-tailed grouse (black) and greater prairie-chicken (gray) fecal pellet samples (left) and hunters harvested birds (right) were collected in Minnesota during 2015, 2016, and 2017. No fecal pellet samples were collected during 2016.

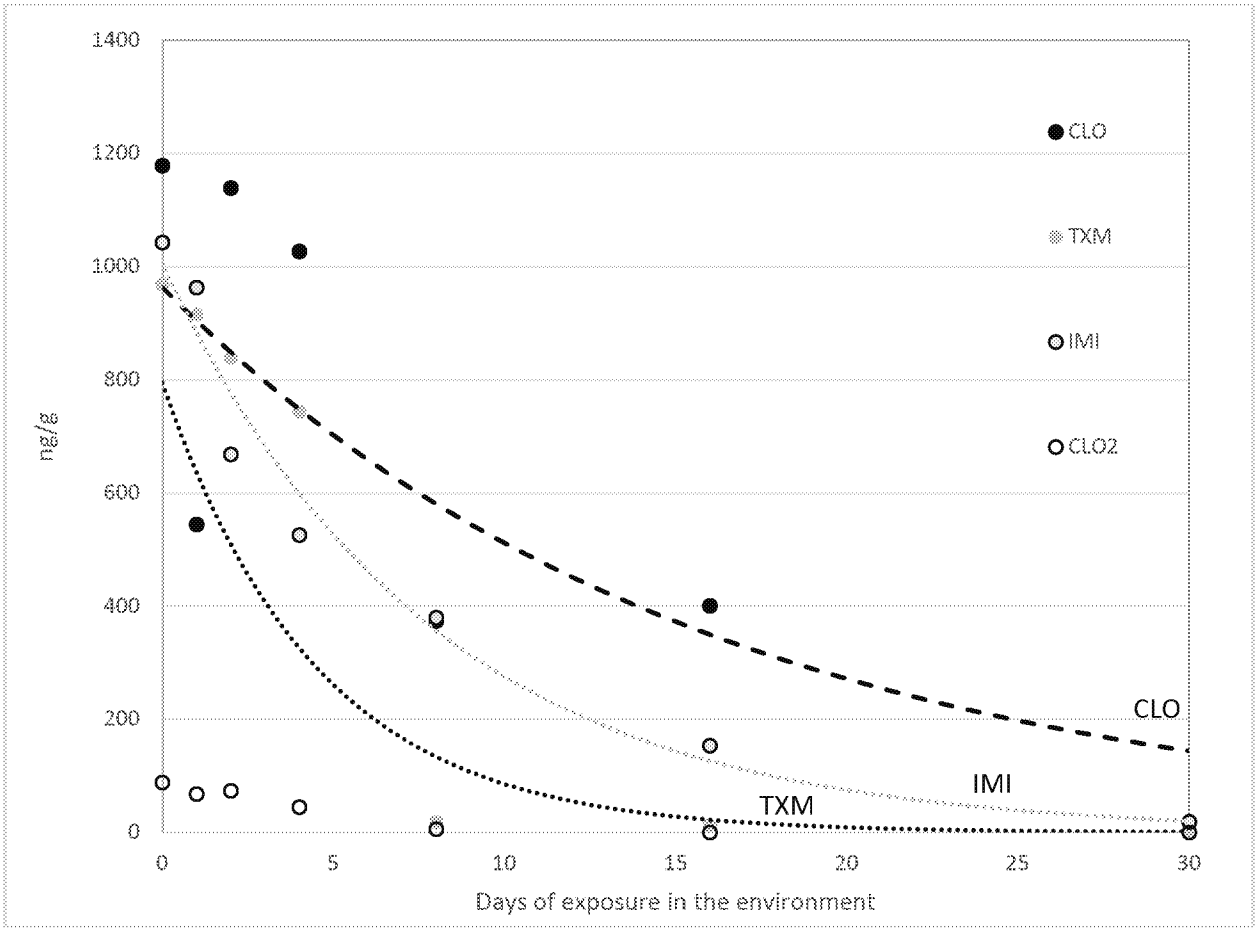

Figure 2. Concentrations of neonicotinoid seed treatments (Clothianidin -CLO, Imidacloprid -IMI, and Thiamethoxam -TXM) on corn and soybean seeds left in the environment on the soil surface for 0-30 days according to an exponential decay model.

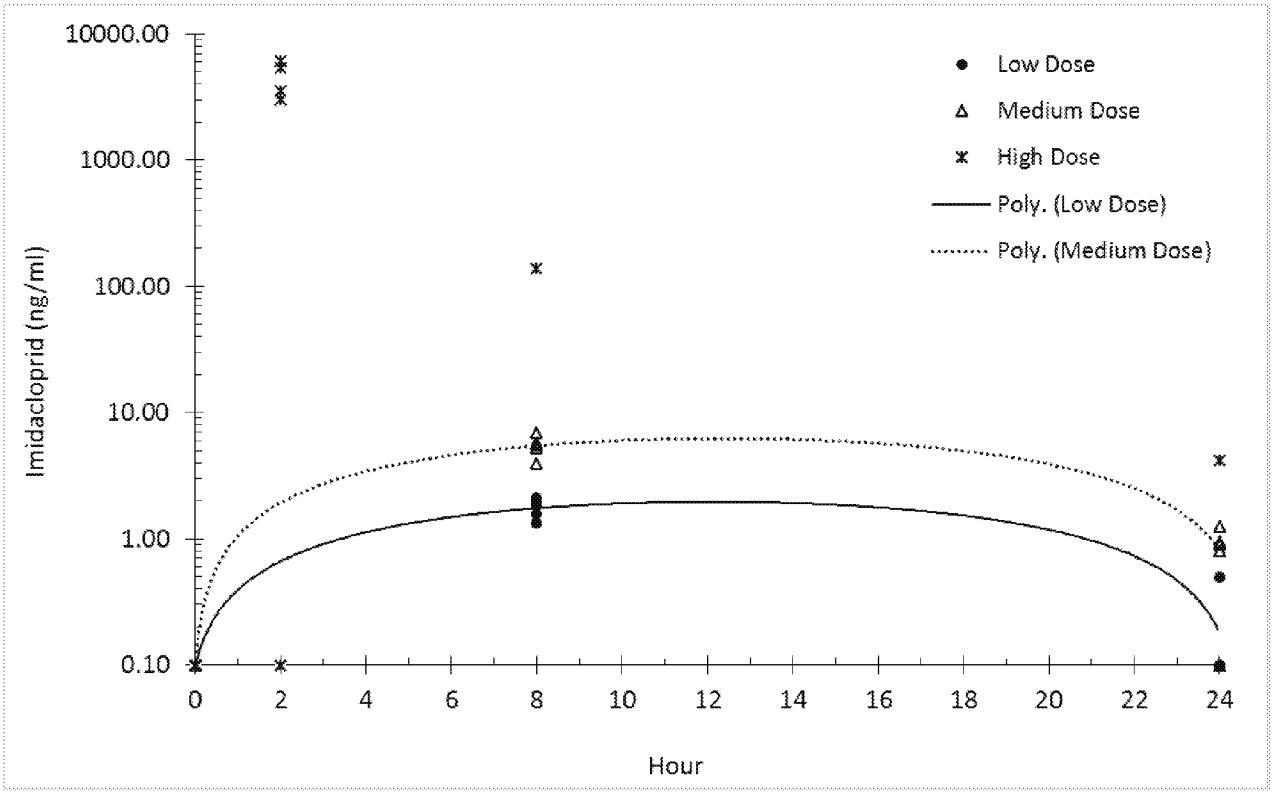

Figure 3. Changes in imidacloprid (IMI) concentrations in blood of dosed chickens over time after one dose. IMI doses were 1%, 5%, and 20% of a reported IMI LD_{50} for chickens (i.e., low, medium, and high dose groups, respectively). IMI detection limit is 0.10 or -1.0 $\log_{10}$ ng/ml in blood. Data 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.

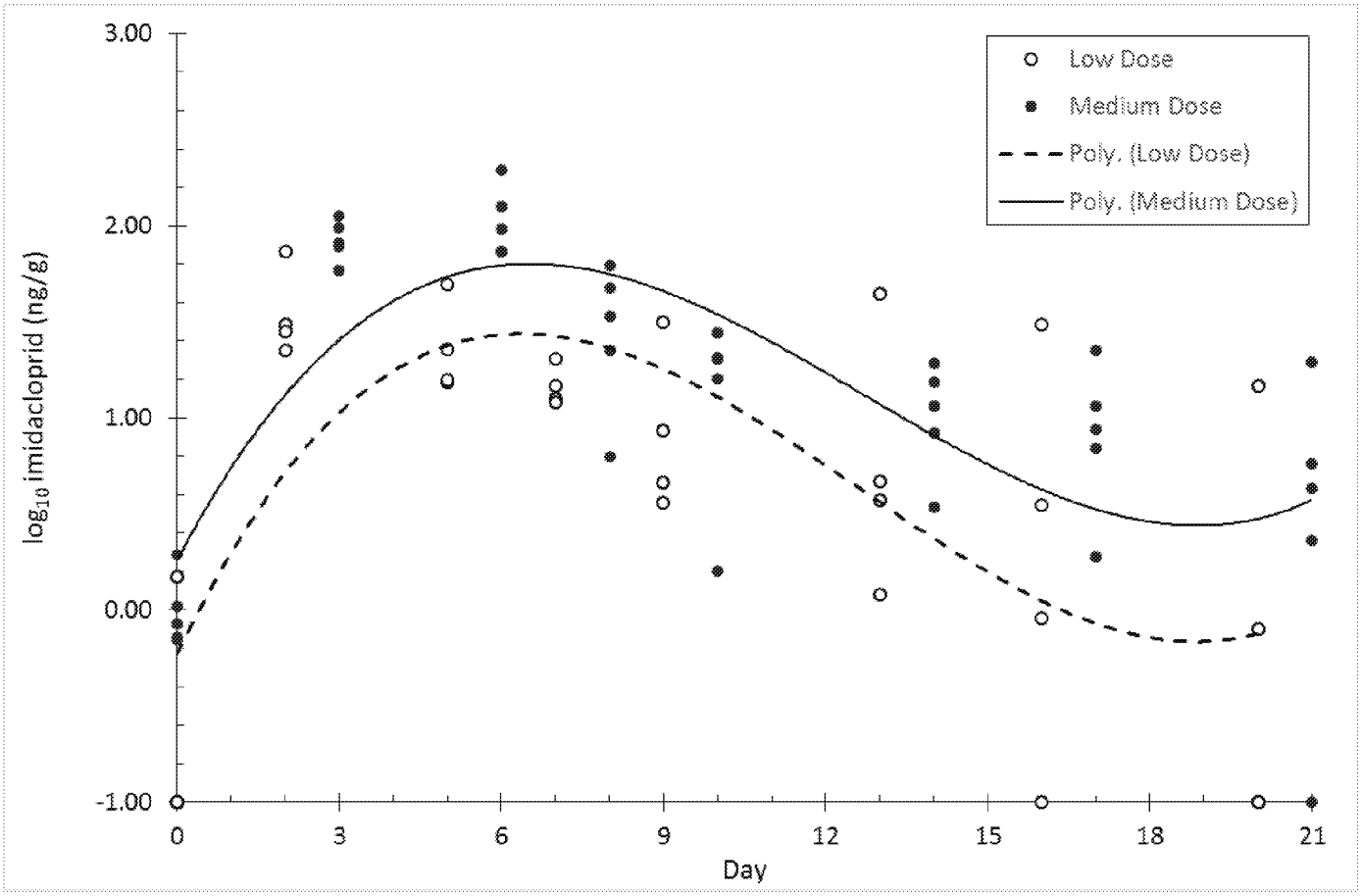

Figure 4. Changes in imidacloprid (IMI) concentrations in feces of dosed chickens over time. Samples collected on day 0 were baseline samples, prior to exposure. Daily IMI dose for 7 days of 1% (low dose) and 5% (medium dose) of a reported IMI LD_{50}. The last day of dosing occurred on day 7 of the 21 day experiment. IMI detection limit is 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.

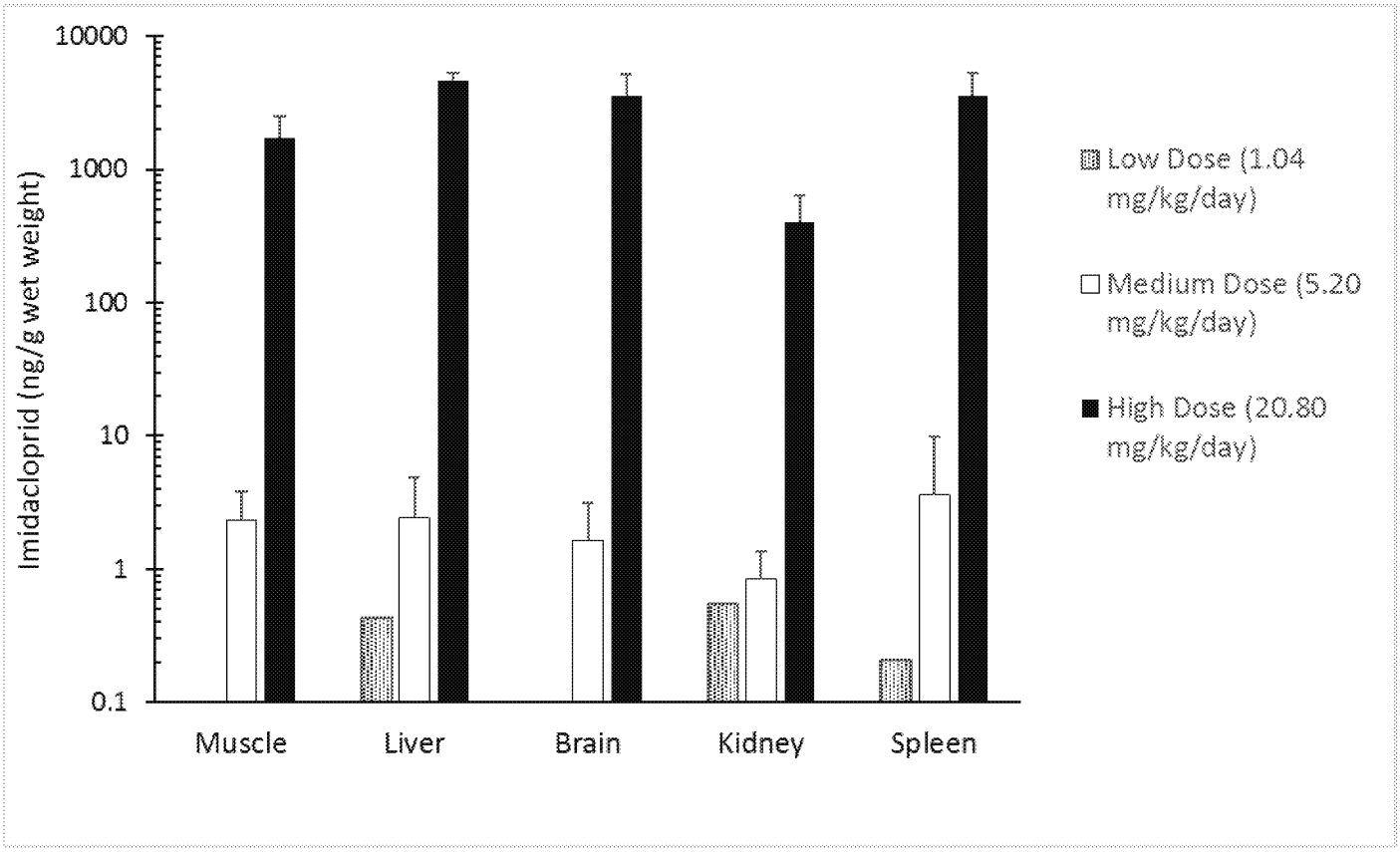

Figure 5. Concentrations of imidacloprid (geometric mean + SD ng/g wet tissue weight) in tissues of laboratory-exposed chickens on experimental day 1 (high dose) or 21 (low and medium dose). Data at the detection limit of 0.10 ng/g are not visible. Error bars represent the standard deviation of observations for a given group. No error bars are provided for the low dose group because bars represent only 1 individual with detectable concentrations.