NEONICOTINOID IMPACTS

A neonicotinoid insecticide reduces fueling and delays migration in songbirds

Margaret L. Eng[1], Bridget J. M. Stutchbury[2], Christy A. Morrissey[3,4]*

Neonicotinoids are neurotoxic insecticides widely used as seed treatments, but little is known of their effects on migrating birds that forage in agricultural areas. We tracked the migratory movements of imidacloprid-exposed songbirds at a landscape scale using a combination of experimental dosing and automated radio telemetry. Ingestion of field-realistic quantities of imidacloprid (1.2 or 3.9 milligrams per kilogram body mass) by white-crowned sparrows (*Zonotrichia leucophrys*) during migratory stopover caused a rapid reduction in food consumption, mass, and fat and significantly affected their probability of departure. Birds in the high-dose treatment stayed a median of 3.5 days longer at the site of capture after exposure as compared with controls, likely to regain fuel stores or recover from intoxication. Migration delays can carry over to affect survival and reproduction; thus, these results confirm a link between sublethal pesticide exposure and adverse outcomes for migratory bird populations.

Birds are frequently exposed to pesticides and other environmental contaminants at migratory stopover sites, but studies that causally link contaminant exposure to migration ability are lacking. Migratory stopover is typically characterized by rapid food intake (hyperphagia) and assimilation of energy stores to prepare for sustained flight (*1*). Suppression of feeding and mass loss has been proposed as a sublethal mechanism through which certain neurotoxicants delay migration and reduce survival (*2*), and disruption of migratory orientation behavior has been identified as a sensitive endpoint of exposure to certain contaminants in songbirds (*3*, *4*). Conditions experienced during migration have population-level consequences (*5*), and thus the presence of contaminants at stopover sites could be contributing to the population declines occurring in many migratory species.

There is increasing concern and controversy over the neonicotinoids, which are the most widely used class of agricultural insecticides worldwide (*6*). Neonicotinoids have a neurotoxic mechanism of action, binding to the nicotinic acetylcholine receptor (nAChR), which causes overstimulation of the nervous system (*7*). They bind more strongly to insect receptors than to vertebrate receptors and were thought to pose a low risk for vertebrates (*7*). However, recent studies have demonstrated that neonicotinoids can have substantial negative effects on survival, condition, and behavior in birds (*8*–*10*). Birds can be exposed through multiple pathways, including contact with sprays, ingestion of contaminated soil and water, as well as consumption of treated seeds. Although neonicotinoids have been on the market since the mid-1990s, researchers have only recently started to focus on identifying field exposures in wildlife, and there is mounting evidence that farmland birds worldwide are routinely exposed to neonicotinoids (*11*–*15*). Bird species that use agricultural habitat for migration or breeding are exhibiting particularly precipitous declines (*16*, *17*). In North America, 74% of farmland-dependent bird species declined from 1966 to 2013, many of which are seed-eaters (*17*). Along with habitat loss and disturbance, the large-scale application of agricultural pesticides, including the neonicotinoid seed treatments, has been associated with these declines (*17*, *18*), but detailed mechanistic studies are needed to establish a causal link.

Despite being an area of great scientific and conservation interest, the influence of neonicotinoids on avian behavior, patterns of movement, and population-level effects remains poorly understood. Birds are particularly susceptible to neonicotinoid exposure during spring migration, which coincides with spring seeding for many treated agricultural crops in the northern midlatitudes (*19*). In a previous captive dosing study, we tested the effects of sublethal imidacloprid exposure on migratory ability in a seed-eating songbird, the white-crowned sparrow (*Zonotrichia leucophrys*), caught at stopover sites during their spring migration. Imidacloprid caused strong acute effects, including rapid body mass loss and migratory disorientation (*10*). To date, researchers have never been able to experimentally track the effects of pesticide exposure on free-living songbirds. New tracking technologies now permit insight into the consequences of neonicotinoid exposure on songbirds at an ecologically relevant scale. In this study, we combined controlled dosing of wild-caught birds (Fig. 1) and automated telemetry to follow fueling and migratory movements of individual white-crowned sparrows experimentally exposed to very low sublethal doses of imidacloprid (3 to 10% of predicted median lethal dose) at a northern stopover in Ontario, Canada. The selected doses were well within the range of concentrations that a bird could realistically consume if they accidentally ingested a few treated seeds (table S1).

We found that even a single exposure to low doses of imidacloprid (1.2 mg·kg body mass^{-1} or 3.9 mg·kg body mass^{-1}, n = 12 birds per treatment) caused negative effects on fueling and migration in sparrows. Imidacloprid-induced mass loss has been reported in previous studies in birds (*8*–*10*), and we observed a significant reduction in white-crowned sparrow body mass 6 hours after dosing [dose*time interaction, linear mixed model (LMM) $F_{2,33}$ = 4.56, P = 0.018] in both the low- and high-dose groups (P = 0.005, average mass loss = 3.0%, and P < 0.0001, average mass loss = 5.9%, respectively) (Fig. 2A and table S2). Control birds subjected to the same procedures did not lose significant body mass (P = 0.156). It is possible that capture and handling may have been sufficient in dosed birds to cause interactive effects with imidacloprid and amplify the magnitude of the dose response. However, even after longer acclimation periods of ~2 weeks, our previous captive study using the same species showed a significant negative effect on body mass after just one comparable dose of imidacloprid (4.0 mg·kg body mass^{-1}·day^{-1}), with birds losing 6.5% of body mass within 24 hours after the first dose and 17% of mass after three consecutive daily doses (table S3) (*10*).

Fat is the essential fuel store in migrating birds (*20*), and body composition quantitative

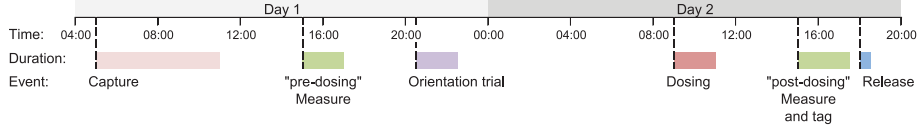

Fig. 1. Experimental timeline for each cohort of white-crowned sparrows captured on migration stopover. The same body measurements were taken ~24 hours apart to compare "pre-dosing" and "post-dosing" conditions. Nocturnal orientation trials tested for baseline migratory activity and orientation, and only birds with fat filling ≥½ the furcular hollow and exhibiting migratory restlessness were screened into the dosing study. Birds were orally dosed the following morning, measured and nanotagged ~6 hours after dosing, and then released 2 hours before sunset. Post-release tracking was accomplished remotely by means of the Motus Wildlife Tracking System.

[1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK S7N 5B3, Canada. [2]Department of Biology, York University, Toronto, ON M3J 1P3, Canada. [3]Department of Biology, University of Saskatchewan, Saskatoon, SK S7N 5E2, Canada. [4]School of Environment and Sustainability, University of Saskatchewan, Saskatoon, SK S7N 5C8, Canada.
*Corresponding author. Email: christy.morrissey@usask.ca

magnetic resonance (QMR) scans confirmed that mass loss in imidacloprid-exposed sparrows was driven by a corresponding loss of body fat (dose*time, LMM $F_{2,33}$ = 3.90, P = 0.030), whereas lean mass loss was similar across dose groups (dose*time, LMM $F_{2,33}$ = 0.16, P = 0.855). Fat content in control birds did not change over time (P = 0.521), whereas fat significantly decreased after dosing in both the low-imidacloprid (P = 0.010; average fat loss = 9.3%) and high-imidacloprid (P < 0.0001; average fat loss = 17.1%) birds (Fig. 2B and table S2). Average lean mass decreased slightly (1.6%) after dosing (time, LMM $F_{1,33}$ = 10.82, P = 0.002) (Fig. 2C and table S2) but was not significantly different between dose groups (dose, LMM $F_{2,33}$ = 2.52, P = 0.096).

The mass and fat loss in white-crowned sparrows appears to be associated in part with "anorexic" effects of imidacloprid. Food consumption during the 6-hour post-exposure period was significantly reduced by imidacloprid (general linear model $F_{2,17}$ = 10.44, P = 0.001), with high-dose birds on average eating 70% less food compared with that of control birds on a body mass basis (P = 0.002) (Fig. 2D). Low-dose birds on average ate 8% less food than did controls, although this decrease was not significant (P = 0.898). Reduced food intake was not related to aversion of seeds because exposure was through oral gavage, and all food provided was untreated. Other avian imidacloprid-dosing studies have similarly observed suppression of feeding behavior (*9*, *21*). It is not clear whether appetite suppression alone is the cause for reduced body mass in exposed birds; other interactive effects of imidacloprid may have altered nutrient assimilation efficiency or caused general toxicity that could exacerbate mass loss. The anorexic properties of nicotine have been associated with central cholinergic-linked metabolic processes (*22*), which provides some insight into the possible mechanistic links between nAChR agonists—such as nicotine and neonicotinoids—and appetite suppression.

We found strong evidence that imidacloprid exposure significantly extended stopover duration (Fig. 3 and tables S4 and S5). The top model that included dose, plus intrinsic (fat and time in captivity) and extrinsic (weather) covariates, had the strongest support and significantly predicted the likelihood of departure (χ^2 = 41.4, df = 5, P < 0.001, n = 33 events), whereas support for the second model without dose was weak [>7 ΔAICc, where ΔAICc is the difference between the model's AICc (Akaike information criteria corrected for small sample size) value and the AICc value of the best fit model] (table S4). For all 33 birds detected at departure, the median stopover duration was 2 days (range 0 to 9 days). The longest stopover durations were observed in dosed birds (control median = 0.5 days, range = 0 to 4 days; low-dose median = 3 days, range = 0 to 8 days; high-dose median = 4 days, range = 0 to 9 days). High-dose birds were only 11.7% as likely to depart under similar conditions as those of control birds [hazard ratio (HR) = 0.117, 95% confidence interval (CI) = 0.024 to 0.453) (table S5).

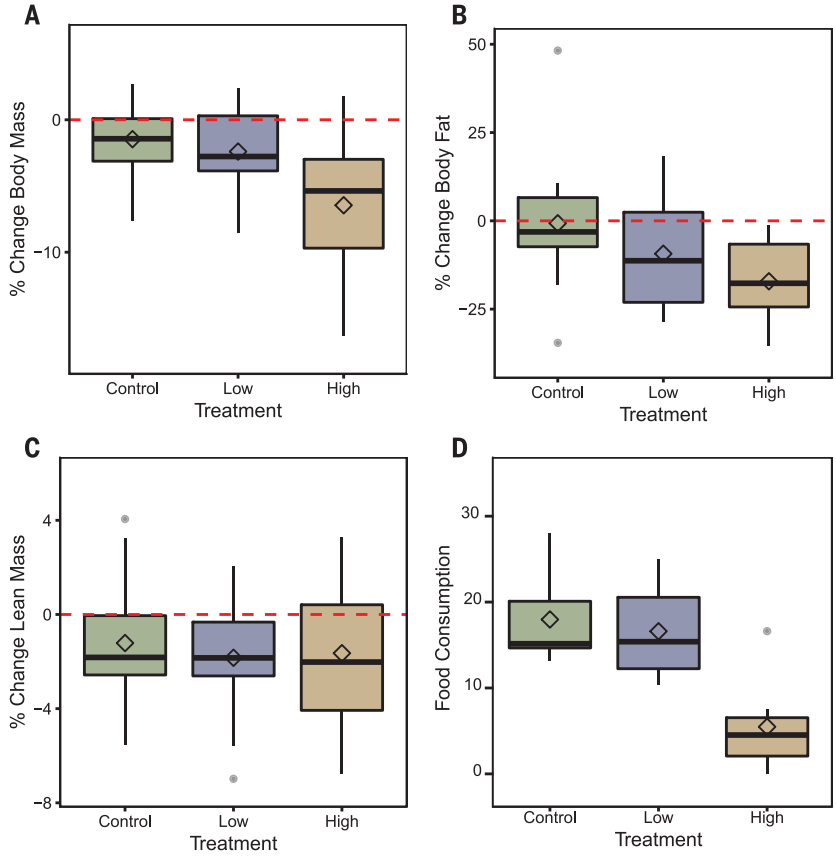

Fig. 2. Effects of a single oral dose of imidacloprid on body condition and food consumption in migrating white-crowned sparrows. (**A** to **C**) Percent change between pre-dosing and 6 hours post-dosing for (A) body mass, (B) fat, and (C) lean mass in white-crown sparrows measured by using QMR. (**D**) Food consumption (gram food·kilogram body mass^{-1}·hour^{-1}) measured over the 6-hour post-dosing period. Control = vehicle sunflower oil; low = 1.2 mg·kg body mass^{-1}; high = 3.9 mg·kg body mass^{-1}. Boxes indicate interquartile range, middle lines indicate median, diamonds indicate the mean, whiskers show the minimum and maximum values within 1.5× the interquartile range, and dots represent outliers (>1.5× interquartile range from box). n = 12 birds per dose group; food consumption is based on averages per cage (one to three birds per cage) within the same treatment.

Low-dose birds were 87.2% as likely to depart as control birds; however, 95% CI for probability of departure closely overlapped with that of controls (HR = 0.872, 95% CI = 0.272 to 2.754) (Fig. 3). We also found that birds that were held longer before being screened into the experiment tended to have shorter stopover duration after release (HR = 2.399, 95% CI = 1.261 to 5.129), and a higher weather index (higher temperature and tailwinds) was associated with a greater probability of departure and a shorter stopover duration (HR = 2.356, 95% CI = 1.514 to 3.995). Predosing fat was not a good predictor of stopover duration, with 95% CIs overlapping one (HR = 1.261, 95% CI = 0.947 to 1.665).

We hypothesize that the extended stopover in imidacloprid-exposed birds was related to the reduced fuel loads and suppression of feeding and fueling ability. Previous studies have found that zugunruhe (migratory restlessness) and the timing of migratory flight are tightly tied with fat-accumulation rate (*23*), and the amount of fuel lost is a better predictor of stopover duration than absolute fuel stores (*24*). Birds that lose more fuel stores during fasting periods tend to have lower zugunruhe once they start refueling, and the motivation to depart is suppressed until sufficient fuel stores for the next flight have been replenished (*24*). It is probable that birds in the high-dose imidacloprid-exposed groups, which lost more fat, would need more time to regain fuel stores, resulting in the longer observed stopovers.

Although captive experiments of white-crowned sparrows exposed to imidacloprid showed disrupted migratory orientation (*10*), we did not find any effects on flight path or orientation in free-living sparrows. There was no statistical difference between the mean overall orientation of any of the dose groups (Watson-Williams $F_{2,27}$ = 1.882, P = 0.172) (Fig. 4), and the mean overall orientation bearing was significantly oriented in a northeast direction (44.1°) for all treatment groups (Rayleigh test, Z_{30} = 28.1, P < 0.0001). Similarly, the departure bearings were consistent across treatments (Watson-Williams $F_{2,27}$ = 1.17, P = 0.326), and the mean departure bearing was slightly more north at 32.6° (Rayleigh test, Z_{30} = 27.0, P < 0.0001). In addition, the average sustained

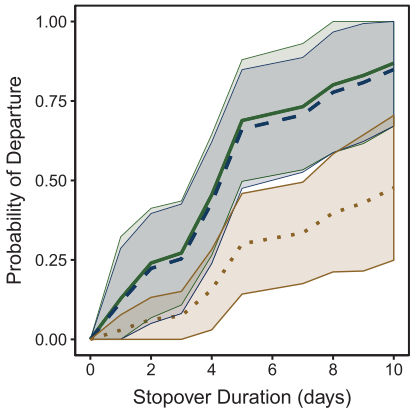

Fig. 3. Effect of a single oral imidacloprid exposure on stopover duration in migrating white-crowned sparrows. Lines indicate predicted probability of departure from the stopover site for each dose group over time. Solid green, control; dashed blue, low dose; dotted brown, high dose; n = 12 birds in control group, 10 birds in low-dose group, and 11 birds in high-dose group. Estimates are adjusted for weather conditions, time in captivity, and predosing fat loads. Shaded area represents 95% CIs, which overlap for the control and low-dose treatments. The probability of departure for high-dose (3.9 mg·kg body mass^{-1}) birds was 8.5 times lower than that of controls (sunflower oil vehicle).

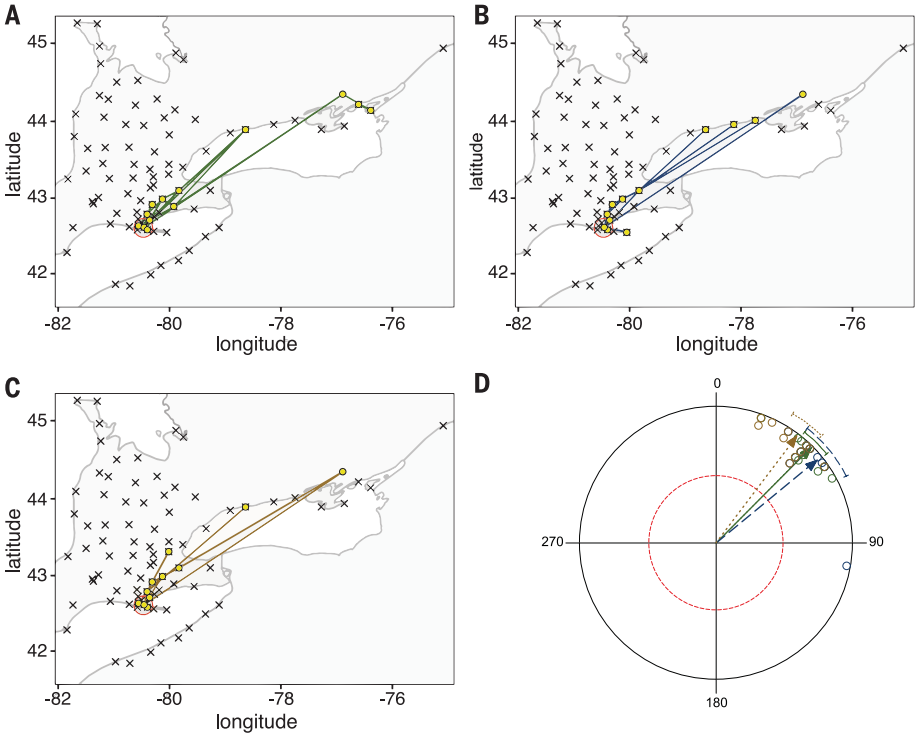

Fig. 4. Migratory flight paths and overall bearings for 30 out of 33 detected white-crowned sparrows tracked in an automated telemetry array in the southern Ontario, Canada. (**A** to **C**) Flight paths of (A) control birds, (B) low-imidacloprid-dose birds (1.2 mg·kg body mass^{-1}), and (C) high-imidacloprid-dose birds (3.9 mg·kg body mass^{-1}). The red circle indicates capture and release site near Long Point, Ontario, and yellow circles indicate tag detections. Lines are drawn between sites with consecutive detections and do not represent actual flight paths, and "×" indicates locations of active radio telemetry receivers during the lifespan of the nanotags. (**D**) Overall bearing of detection paths. Solid green, control; dashed blue, low dose; dotted brown, high dose. Open circles represent the bearing of individual birds, and arrows represent mean orientation of each treatment group; the length of the arrows indicates how closely individuals are clustered around the mean, the dotted red line indicates the critical values for Rayleigh's uniformity test at $\alpha = 0.05$ (vectors that pass this critical value are significant), and the outer arc represents the 95% CI for each significant vector.

migratory flight speed was similar across treatment groups (20.1 ± 1.6 m·s^{-1}, LMM $F_{2,23}$ = 0.29, P = 0.753).

The lack of effects on orientation and speed in our field study suggest that free-living birds avoid migratory flight while recovering from intoxication. Imidacloprid nAChR binding is reversible (*7*, *25*), and birds are able to recover from nonlethal exposures. In the previous captive study, birds tested in orientation funnels regained both body mass and orientation ability within 2 weeks after the completion of dosing (*10*). The extended stopover we observed in birds exposed to a single high dose likely served as a self-imposed recovery period to regain fuel stores or recover from neurotoxic effects.

Extended stopovers while intoxicated and in reduced body condition could lower survival by increasing susceptibility to predation or inclement weather. Imidacloprid exposure could also reduce fitness through the sublethal consequences of longer stopovers. There are selective pressures for birds to minimize the time spent migrating (*26*). Birds that are delayed during migration and arrive later at breeding grounds have been reported to obtain poorer-quality territories, breed later, and produce fewer offspring in worse condition than those of early arrivals, reducing the probability of their offspring recruitment to the population (*27*, *28*). The seed-treatment application rates of common neonicotinoids (including imidacloprid) for several crops are at concentrations at which only few treated seeds (<5) need to be consumed to reach the level of concern for acute lethality in small- and medium-sized birds (*29*). Treated seed avoidance has been documented but is a learned response rather than innate sensory aversion to neonicotinoid seed coatings and so offers little protection to naïve birds (*30*, *31*). Birds will consume spilled or near-surface seeds after planting as a food source (*11*, *15*), and there are reports of free-ranging birds consuming enough treated seeds to reach lethal concentrations (*14*, *32*). The widespread use of neonicotinoids along migratory routes in the United States and southern Canada means that individuals may experience repeated exposure at successive stopover sites, resulting in cumulative delays, which would amplify these negative fitness consequences.

This is the first time researchers have been able to track the fate of free-living pesticide-exposed birds over ecologically relevant spatial scales. Through the use of controlled dosing and automated telemetry, we were able to discern that improper flight paths caused by disorientation are likely not the main concern for imidacloprid exposure because birds appear to avoid flight while intoxicated or regaining body fat. Instead, the neonicotinoids act as anorexic agents, causing mass loss during a critical life stage that is typically characterized by hyperphagia and rapid fat accumulation. In addition to directly decreasing survival when consumed at acutely toxic concentrations (*14*, *32*), ingestion of minute sublethal quantities of imidacloprid during the critical migratory stopover period causes delays that could also reduce future fitness. The sublethal effects of imidacloprid on food consumption, body condition, and stopover duration have clear links with survival and reproduction and are predicted to negatively affect populations of migratory birds that commonly use agricultural habitats for refueling.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Tables S1 to S5
References (*34*–*50*)

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Science

A neonicotinoid insecticide reduces fueling and delays migration in songbirds

Margaret L. Eng, Bridget J. M. Stutchbury and Christy A. Morrissey

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Hazardous delays

Neonicotinoids are a widely used group of pesticides that have been shown to have negative impacts on an increasing number of species, most notably pollinators. Eng *et al.* tested how exposure to these compounds influenced the behavior of a migrating songbird. Ingestion of field-realistic levels of neonicotinoid insecticides reduced feeding and accumulation of body mass and fat stores, which led to delayed departure from stopover sites. Such delays can lead to reduced migration survival and decreased reproductive success and therefore have the potential to impose population-level impacts.

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