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From seeds to plasma: Confirmed exposure of multiple farmland bird species to clothianidin during sowing of winter cereals

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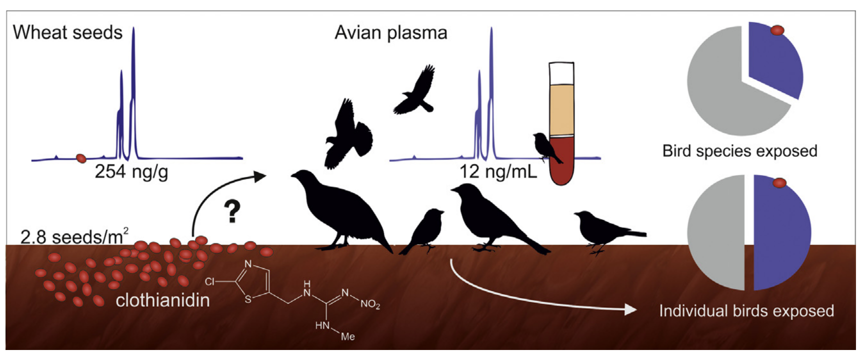
HIGHLIGHTS

- Exposure of farmland birds to a neonicotinoid seed treatment was characterised.
- Treated cereal seeds were found on the soil surface at all 25 farms surveyed.
- 15 species of bird were observed consuming clothianidin-treated seed at seed piles.
- Clothianidin was detected in the plasma of 10/11 farmland bird species sampled.
- Birds consumed up to 65% of a chronic toxicity estimate for clothianidin.

GRAPHICAL ABSTRACT



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ABSTRACT

Neonicotinoids are the largest group of systemic insecticides worldwide and are most commonly applied as agricultural seed treatments. However, little is known about the extent to which farmland birds are exposed to these compounds during standard agricultural practices. This study uses winter cereal, treated with the neonicotinoid clothianidin, as a test system to examine patterns of exposure in farmland birds during a typical sowing period. The availability of neonicotinoid-treated seed was recorded post-sowing at 39 fields (25 farms), and camera traps were used to monitor seed consumption by wild birds in situ. The concentration of clothianidin in treated seeds and crop seedlings was measured via liquid chromatography-tandem mass spectrometry, and avian blood samples were collected from 11 species of farmland bird from a further six capture sites to quantify the prevalence and level of clothianidin exposure associated with seed treatments. Neonicotinoid-treated seeds were found on the soil surface at all but one of the fields surveyed at an average density of 2.8 seeds/m^2. The concentration of clothianidin in seeds varied around the target application rate, whilst crop seedlings contained on average 5.9% of the clothianidin measured in seeds. Exposure was confirmed in 32% of bird species observed in treated fields and 50% of individual birds post-sowing; the median concentration recorded in positive samples was 12 ng/mL. Results here provide clear evidence that a variety of farmland birds are subject to neonicotinoid exposure following normal agricultural sowing of neonicotinoid-treated cereal seed. Furthermore, the widespread availability of seeds at the soil surface was identified as a primary source of

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exposure. Overall, these data are likely to have global implications for bird species and current agricultural policies where neonicotinoids are in use, and may be pertinent to any future risk assessments for systemic insecticide seed treatments.

1. Introduction

Since their introduction in the early 1990s, neonicotinoid (NN) insecticides have grown in use and by 2014 accounted for approximately one-third of the insecticide market worldwide (Simon-Delso et al., 2015). NNs are the most widely used class of systemic insecticide in agricultural practice, consisting of seven commercially available compounds that are applied in >100 countries (Simon-Delso et al., 2015). The most commonly used (in descending order) are thiamethoxam, imidacloprid and clothianidin, which are predominantly applied as seed treatments and have been registered for use on >140 crops worldwide (Simon-Delso et al., 2015). NNs act as agonists for the nicotinic acetylcholine receptors in the central nervous system of invertebrates, causing paralysis and death (Tomizawa et al., 2000), and are designed to be taken up into xylem and distributed throughout the plant to provide long-term protection. It was assumed that these factors would predispose NNs to being less of a risk to many non-target species compared to older insecticides (Simon-Delso et al., 2015). However, in 2018 the EU banned the outdoor use of imidacloprid, thiamethoxam and clothianidin (Implementing Regulations 2018/783, 784 and 785) on the basis of a review of risks to bee health (Bass and Field, 2018). Subsequently, concerns have also been raised regarding their potential effect on other non-target species, particularly farmland birds (Pisa et al., 2014; Goulson, 2013; Wood and Goulson, 2017).

Over the last 50 years, farmland birds have undergone substantial population declines across Europe and North America and much of this has been attributed to agricultural intensification (Stanton et al., 2018; Donald et al., 2001); this will include habitat loss, seasonal shifts in cultivation practices, and greater use of block cropping, but also an increase in the proportion of rural landscapes that is treated with pesticides. Historically there has been evidence of insecticides adversely affecting birds both directly (e.g., the effect of the organochlorine DDT on birds of prey (Newton, 1979)), as well as indirectly (e.g., decreased food availability during the breeding season as a result of broad spectrum insecticides (Boatman et al., 2004)). However, the effect of systemic insecticides (such as NNs) on individual birds in the field is largely unknown. Relative toxicity to birds differs markedly between NN compounds (Tomizawa and Casida, 2005); for example the acute dose that is lethal to 50% of the test population (LD_{50}) for bobwhite quail *Colinus virginianusis* is 152 mg/kg/body weight for imidacloprid, but >2000 mg/kg/body weight for clothianidin (European Food Safety Authority, 2006). In aviary conditions, NNs are known to cause sub-lethal effects in birds, such as adverse impacts on the reproductive system (Lopez-Antia et al., 2013; Pandey and Mohanty, 2015; Mohanty et al., 2017; Pandey and Mohanty, 2017), alterations to the immune system (Lopez-Antia et al., 2013), neurotoxic symptoms (Addy-Orduna et al., 2018; Rawi et al., 2019), oxidative stress (Zeid et al., 2019) and changes to behaviour (Eng et al., 2017; Cox, 2001); many of these sub-lethal effects have been reported at environmentally-relevant doses in laboratory studies using wild bird species. Furthermore, Mineau and Palmer (2013) estimated that the ingestion of 1.3 imidacloprid- or 4.4 clothianidin-coated wheat seeds would be sufficient to breach adverse reproductive end points in a small (15 g) songbird, based on a typical seed loading of 0.033 and 0.025 mg/g of imidacloprid and clothianidin, respectively (Mineau and Palmer, 2013).

Many farmland birds have a high proportion of agricultural seeds and plant material in their diet (Holland et al., 2006), and so have potential for exposure to NNs applied as seed treatments through ingestion of either treated seed or seedlings (Mineau and Palmer, 2013). The presence of pesticide active substances on potential food items highlights the need for quantitative risk assessment as part of the authorisation process, but the complexity of undertaking this assessment where direct and substantive exposure can be expected is widely acknowledged. The risk from dietary exposure is assessed within regulatory procedures by combining information on toxicity of the respective compound and estimates for levels of exposure that wild birds may be subject to via seed treatments (European Food Safety Authority, 2010). Lower tiers of assessment will normally indicate potential risk for seed treatments, so 'higher tier' assessment, such as the use of radio-tracking data and focal species dietary data, is required to estimate the level of exposure in farmland bird species more accurately (European Food Safety Authority, 2006). Product application instructions also play an important part in safeguarding wildlife from pesticide use. With regards to NN seed treatments specifically, product labels state that seeds should be buried at a minimum depth of 4 cm, and that no seed should be left on the soil surface after drilling (Bayer Crop Science UK, 2019). Nevertheless, it is recognised that absolute compliance with this requirement is impossible to achieve (European Food Safety Authority, 2010). The availability of seed on the soil surface is known to be a function of crop type, season of sowing, soil condition, sowing equipment, and location within the field; for example, in the Netherlands, average surface seed densities were greatest for winter wheat with standard sowing equipment (20 and 31 seeds/m^2 measured in successive seasons) and on average there was 3.5 times more seed present on the soil surface at the headlands compared to the field centre (de Snoo and Luttik, 2004).

To date, only a handful of studies have collected field data to investigate exposure of wild birds to agricultural seeds treated with NNs. Studies in Canada and the US investigated the availability of treated seed on the soil surface after drilling (McGee et al., 2018; Roy et al., 2019), whilst the US study also measured NN concentrations in treated seed collected from the field and reported highly variable concentrations among three seed types and three NN compounds (Roy et al., 2019). Several papers report that NN uptake in crops is highly variable (Li et al., 2018a; Balfour et al., 2016), such that residue taken up by seedlings can vary between 1 and 15% of that in treated seeds (Prosser and Hart, 2005; Alford and Krupke, 2017); for comparison, the European Food Safety Authority recommends a default within its regulatory assessment of 20% of the seed treatment being taken up by seedlings (European Food Safety Authority, 2010). In terms of exposure of birds to NNs, a US study documented 10 confirmed bird species and various unidentified sparrow species feeding at experimentally-placed treated seed piles (Roy et al., 2019), and a UK-based report observed 18 bird species feeding on the types of crop seed that could be treated with NNs, again using experimentally-placed seed (Prosser, 2001). A study in Spain observed 30 species of wild bird consuming pesticide-treated seed in newly sown fields, and reported imidacloprid exposure in partridges that was not associated with the seed treatments (Lopez-Antia et al., 2016).

NN residues have been measured in a range of avian samples, but only from a limited number of farmland species. Thus far, NNs have been detected in the liver, crop or eggs of four gamebird and three columbid species (Bro et al., 2016; Millot et al., 2017; Ertl et al., 2018; Turaga et al., 2016). NN poisonings have also been documented in grey partridge *Perdix perdix* and columbid species, of which >70% (of 101 incidences and 734 mortalities) occurred during the autumn sowing period (Millot et al., 2017). Only three studies have measured concentrations of NNs in avian plasma: two in raptor species (Byholm et al., 2018; Taliansky-Chamudis et al., 2017), and one in a songbird species (Hao et al., 2018), with 38 out of 76 individuals testing positive for NNs across the three separate datasets. Most recently, a large study in Switzerland measured NN concentrations in the feathers of house sparrows *Passer domesticus* and found 100% prevalence of NNs (consisting of five compounds) in 146 pooled samples collected from 62 farms across the Swiss plateau (Humann-Guilleminot et al., 2019a).

Thus, there is evidence that a range of farmland birds have the potential to be exposed to NN treated seeds and that residues of NNs can be detected in biological samples taken from birds. To link these lines of evidence together and form an understanding of the entire exposure pathway for seeds treated with a NN, we conducted a field-based study that investigated patterns of clothianidin (CLO) exposure within a typical farmland bird community, via treated winter cereal seeds sown according to standard agricultural practice. CLO was selected as the main NN used in treatment of winter cereal seeds in the UK at the time of the study (37% of the 22,600 km^2 of winter wheat grown in 2016 received a CLO seed treatment; (Garthwaite et al., 2013). The objectives of the study were to: 1) measure availability of treated seeds on the soil surface after sowing; 2) quantify CLO concentrations in treated seeds and seedlings collected from the field; 3) identify avian species that may be exposed to CLO in recently sown cereal fields; and 4) monitor avian blood plasma for CLO contamination in samples collected from multiple bird species pre- and post-sowing.

2. Methods

Data were collected from 45 fields distributed across a total of 31 farms located in the regions of East Anglia (UK) and North Lincolnshire (UK) during the autumn sowing seasons of 2015 (21 fields), 2016 (18 fields) and 2017 (6 capture sites adjacent to treated fields). These were the two regions in the UK that annually received the greatest mass of CLO applied as seed treatments (Garthwaite et al., 2013); the regions are dominated by arable agriculture with winter cropping rotations (winter cereals with break crops of oilseed rape or field beans). Seed, seedling and bird survey data were obtained from fields in East Anglia (only one field on a farm in any year), whilst avian blood plasma samples were obtained from capture sites adjacent to treated fields in Lincolnshire once licenses for the procedure had been granted. Farms were selected using existing contacts of conservation and research staff at the Royal Society for the Protection of Birds, supplemented by asking those farmers for additional local contacts. Fields were then selected that were remote from landscape features that would strongly influence bird abundance, such as woodland, tall trees along the margin, or gamebird cover. Farmers were aware that researchers would be surveying fields after drilling, but maintained their standard practice seedbed preparation and drilling methods. Tables S1A and S1B give full details on characteristics of all sampling locations as well as information on all visits for sample collection.

2.1. Density of seeds on the soil surface

Surveys of seed density (expressed as number of seeds per m^2 of soil surface) were conducted across 39 fields in East Anglia sown with CLO-dressed wheat seeds (either Redigo Deter® or Deter®; Bayer PLC, UK). All seed treatment was carried out either by the seed supplier company when purchased or a specialist seed treatment contractor who visited farms to do the work. In total, 21 fields were surveyed in autumn 2015 and 18 fields in autumn 2016. Surveys took place for up to two weeks post-sowing (on or as close as possible to days 1, 3, 6, 9 and 12, where day 0 was within 24 h of sowing). At each visit (Table S1A), the number of treated wheat seeds visible on the soil surface was recorded in 60 quadrats (0.25 m^2), comprising 20 quadrats in the field centre and 20 quadrats at each of two field headlands; quadrats were evenly distributed along transects diagonally bisecting the headland and field centre, so spacing between quadrats was greater on larger fields.

2.2. Treated seed & seedling sample collection

CLO-treated wheat seeds present on the soil surface were collected during seed density surveys (1–14 days post-sowing) from 24 fields in East Anglia. NN-treated wheat seedlings were collected from 20 fields; whole seedlings inclusive of roots and shoots (as extracted from soil) were collected in weeks 2–4 and 5–13 post-sowing, covering two stages of wheat growth (small seedlings at growth stage 10–16, and early tillering plants at growth stage 20–26 (Meier, 1997)). The seeds did not include husks as standard practice is to remove husks before NN application; the presence of the outer cuticle of the seed was not systematically recorded because it became impossible to distinguish the cuticle as it decomposed.

Pooled samples, generally comprising of up to 10 individual seeds or seedlings were collected from the field centres and headlands of each field on each sampling occasion to assess inter-site variation in CLO concentrations; samples were collected at locations immediately adjacent to transects for measuring surface seed density. In addition, individual seeds and seedlings were collected and analysed separately from four fields per sample type to assess intra-site variation in CLO concentrations (Table S1A). Results from these individual samples were recombined into a pooled sample for inclusion within statistical analyses that drew on data from all fields. Seed and seedling samples were stored at −20 °C until analysis.

2.3. Bird abundance surveys & camera trap data

Bird surveys were undertaken on the same fields where surface seed densities were recorded. Surveys were undertaken in the months of September to December 2015 and 2016, at the same sampling time points used to assess surface seed densities (on or as close as possible to day 1, 6, 9, and 12), and at a further two time points (2–4 weeks and 5–13 weeks post-sowing) to coincide with seedling collection. Birds utilising treated fields were recorded by: 1) scanning the entire field on arrival and counting the number of birds present; 2) walking along, and counting birds in field boundaries; and 3) flush counts whilst walking field transects (maximum of three transects per field separated by at least 100 m). The location (field boundary, centre or headland) and number of each species observed were recorded, excluding birds flying over the site. The locations of any seed clusters (defined as >10 seeds within a 5 × 5 cm^2 area) that were spilt during standard agricultural practice were also noted as part of these surveys. Motion-sensitive infrared camera traps (Bushnell, USA) were placed at 40 of the larger seed clusters (>100 seeds each) across 21 fields (Table S1A) and remained active until seeds were depleted. Cameras recorded bird feeding activity by recording 10 s of continuous video footage when triggered by movement. On average, each camera recorded data for 4.2 days (range: 1–9 days); cameras were active between 1 and 21 days post-sowing. Camera footage was processed to obtain the following for each bird observed: species, time and date of observation, time at seed cluster and the number of NN seeds (visually identifiable by red dye) consumed. The cameras were very sensitive to movement and almost always continued filming immediately during an active feeding event. A conservative protocol for inferring continued consumption of seed by the same individual bird was adopted, based on minimal gap in filming (generally 0 s, but maximum 2 s) and certainty from the film processor that they identified the same individual bird both within and across successive clips. When it was no longer certain that the same individual was involved (including within a single clip), the seed consumption figures were recorded as two separate observations so seed consumption data are likely to underestimate actual behaviour.

CLO exposure estimated from the mean and maximum number of seeds consumed by each species (per visit) and the mean concentration of CLO measured on treated seed, was compared to the no-observed adverse effect-level (NOAEL) for chronic exposure and lethal dose for 50% of individuals (LD_{50}) for acute exposure to CLO in tested species of birds. These toxicity endpoints were adjusted to account for differences in body weight by taking the value given by Mineau and Palmer (2013) for a 15 g bird at the 5% tail of sensitivity, and scaling to the average weight of each species included in the present study (Robinson, 2005).

2.4. Collection of avian plasma samples

Plasma samples were obtained from birds captured at sites in North Lincolnshire that were immediately adjacent to fields that had been drilled with CLO-dressed wheat seeds treated with Redigo Deter® (one site was within 50 m of the treated field; Table S1B). Capture sites were separated by an average of 22 km (range: 5–40 km) to ensure spatial independence. Birds were sampled across the six capture sites at two time points between September and November 2017: pre-sowing, before any treated seed was drilled at each farm (temporal control group), and within 2 weeks after fields were drilled (temporal treatment group). Pre-sowing was defined as being before treated seeds were drilled on the farm adjacent to the capture site (Table S1B); as such, we cannot rule out that birds could be exposed during the pre-sowing period if other farms in the wider landscape had already drilled treated seed. Birds were caught between sunrise and midday, using up to 66 m of mist-nets per visit situated along field boundaries. Birds were extracted from mist-nets and processed following standard British Trust for Ornithology procedures (species, age, sex and bird weight recorded where possible (Redfern and Clark, 2001)). Blood was taken from designated species via brachial venepuncture under Home Office licence (Table S2). The maximum amount of blood taken from any bird was equal to 1% of its body mass. A health check took place prior to blood being taken, and again prior to release. Blood was collected and stored on ice in heparinised haematocrit tubes and centrifuged (1000 rpm, 5 min) within 3 h of collection. Samples were stored at −20 °C until they were analysed.

2.5. Residue analyses

In total, 111 seeds (73 pooled, 38 individual samples), 93 seedlings (32 pooled, 61 individual samples), and 96 plasma samples from individual birds were analysed for CLO using liquid chromatography-tandem mass spectrometry (LC-MS/MS; see Supplementary Note 1 for extraction and LC-MS/MS method details). Three protocols were used during each LC-MS/MS batch run for quality control and assurance purposes: 1) a deuterated internal standard was added and analysed in all samples; 2) all batches contained a matrix-matched blank which was analysed for CLO and the deuterated internal standard; and 3) during analytical runs a traceable National Institute of Standards and Technology certificated standard (Clothianidin; SPEX, London, UK) was also analysed. The performance of the method was assessed for accuracy (recovery of the internal standards from all samples) and consistency (between-batch analyte linearity). Recovery for the total procedure was calculated using the labelled standards and all residue data were recovery corrected. Ten samples (2 seed, 5 seedling, 3 avian plasma samples) with recoveries <60% and >120% were excluded from subsequent data analyses. The mean (±SE) recoveries for the remaining samples were 99.9 ± 0.9% for seeds, 103.9 ± 1.2% for seedlings and 82.7 ± 1.6% for avian plasma. The limit of detection (LOD) and limit of quantification (LOQ) for clothianidin were 0.4 ng/g and 0.6 ng/g, respectively for seeds and seedlings, and 0.15 ng/mL and 0.21 ng/mL, respectively for plasma samples. The LOD was determined using three-times the signal-to-noise ratio, and the LOQ was calculated as the LOD plus the calculated expanded uncertainty of the method. The expanded uncertainty for CLO was calculated using the Nordtet TR537 handbook (Magnusson et al., 2012). With regards to avian plasma samples specifically, there was no significant difference in the recoveries between samples of differing volumes (20–50 μL; Kruskal-Wallis: $\chi^2_3 = 1.15$, $p = 0.763$).

2.6. Dissipation of clothianidin on treated seeds

The residue of CLO on treated seeds exposed at the surface will decrease over time in response to any wash off due to rainfall or abiotic degradation such as photolysis. The rate of this decrease was described by deriving a first-order dissipation half-life (DT_{50}, days) for CLO on seed samples. First-order reaction kinetics were fitted to the change in concentration of CLO on seeds over time across all seed samples using Eqs. (1) and (2), where C_0 and C_t are the concentrations of CLO in each sample at time 0 and time (t), respectively, t is the number of days post-sowing at which the sample was collected, and k is the first-order rate constant.

$$C_t = C_0.e^{-kt} \quad (1)$$

$$DT_{50} = \frac{\ln(2)}{k} \quad (2)$$

2.7. Statistical analysis

Due to the heterogeneity of the data, spatial and temporal patterns of CLO exposure were analysed using a combination of non-parametric tests and generalised linear mixed models (GLMMs; Table 1). The fit of all GLMMs was assessed by measuring over-dispersion and the visual and statistical assessment of modelled versus simulated residuals. All models were also tested for zero-inflation and inter-correlation between fixed effects. All GLMMs except for those modelling surface seed density were run using a negative-binomial distribution to account for over-dispersion (surface seed density was run using a Gaussian distribution). All analyses were conducted using R (R Core Team, 2013) and the package 'glmmTMB' (Brooks et al., 2017).

Two GLMMs were used to analyse parameters related to surface seed densities and seed and seedling residue data (Table 1). The variable 'number of days post sowing' was structured such that all data points were categorised into the following five groups: 0–1, 2–4, 5–7, 8–10 and 11–14 days post-sowing and treated as an ordered factor within the model. The variable 'cumulative rainfall' referred to the amount of rain that fell at each field, in each year between the date of sowing and date of sample collection. Rainfall data were collected from weather

Table 1
Summary of models used for data analyses.

Response variable	Fixed effects	Random effect
Surface seed densities		
Log(mean surface seed density per field, per survey +1)	Location within field + N days post-sowing + year	(1|field)
Seed and seedling residue data		
CLO residue in seeds	Cumulative rainfall (between sowing and sample collection) + N days post-sowing	(1|farm)
Bird abundance data		
Bird abundance (per survey, per taxonomic guild)	Mean surface seed density (per field, per survey)	(1|field)

Mean surface seed density was calculated for each field and at each field location (headland and field centre).
CLO: clothianidin; N: number of.

stations in the Met Office MIDAS network (Met Office, 2012), and matched to fields based on the geographical proximity (usually within 1.6 km). Field and farm were included as random effects to account for both field-specific influences, and any more general locational effects associated with sampling different fields from the same farm in successive years. In these analyses, all 'pooled' residue data for seed and seedling samples included the concentration of CLO obtained from samples analysed as a pool of items (one data point per pool), and data for samples analysed as individual items (one mean data point per group of individual samples collected at the same field and on the same date). For statistical analyses relating to the burden of CLO in any sample item (seed or seedling), the total mass of CLO in any pooled sample was divided by the number of sample items in that pool.

To analyse bird abundance as a function of surface seed density (Table 1), the mean surface seed density was calculated per field per survey event and assigned to each bird abundance record (total number of each species observed in each field at each survey event). Published dietary data (Cramp, 1985) were then used to determine whether agricultural seed is present or absent in the diet of species observed, as a means of refining the species groups included in each avian GLMM model. Specifically, models were run for those species where agricultural seed was deemed as 'present' in the diet (i.e. those species where the term 'crop grain' – or a specific seed to which NNs are known to be applied, such as plants of the genus: *Beta*, *Triticum*, *Hordeum*, *Linum*, *Secale*, *Brassica*, *Avena* were included in the list of known food items; Table S3) and for those species where agricultural seed was 'absent' from the diet (those species where the previously mentioned terms were not included in the available dietary data; Table S3). Published data were used as the basis for this dietary categorisation to standardise the approach, but this benefit is offset by the potential inclusion of species where grain is a relatively small proportion of the total diet. A number of additional models (each representing a specific taxonomic guild; Table S3) were then run using a subset of species where agricultural seed was deemed to be present in the diet. For each multi-species analysis, the dependent variable was the aggregate count summed across species.

3. Results

3.1. Surface seed densities

Seeds were present on the soil surface in 38 out of 39 fields surveyed in autumn 2015 and 2016, and in 20% (1804/8930) of quadrats; the number of seeds recorded at the soil surface across all quadrats ranged between 0 and 364 seeds/m^2, with a mean of 2.8 ± 12 (SE) seeds/m^2. In addition, the presence of seed clusters (>10 seeds within a 5 × 5 cm^2 area) was confirmed at 31 out of 39 fields across the two years.

There was a significant difference in surface seed densities between fields (Kruskal-Wallis: $\chi^2_{38} = 101.6$, $p < 0.001$); the mean (± SE) number of seeds at each field ranged from 0.11 ± 0.07 to 12 ± 2.5 seeds/m^2. The mean density of seeds on the soil surface after drilling was found to be higher at field headlands (3.7 ± 0.36 seeds/m^2), compared to field centres (0.9 ± 0.06 seeds/m^2; Fig. 1). Mean surface seed density was found to decrease significantly with the number of days post-sowing and was positively associated with location on the headland (versus field centre) (Table 2).

3.2. Clothianidin residue: seed & seedling samples

3.2.1. Seeds

The concentration of CLO recorded in pooled seed samples collected within 2 weeks post-sowing varied between 0.01 and 550.9 μg/g. However, the CLO residue recorded in pooled seeds did not significantly differ between fields within the first 24 h post-sowing (Kruskal-Wallis: $\chi^2_{19} = 21.9$, $p = 0.288$), or during the entire study period (Kruskal-Wallis $\chi^2_{23} = 31.1$, $p = 0.120$). CLO residue in pooled seeds decreased with the number of days post-sowing (Table 2) with a dissipation half-life of 4.2 days (Fig. 2A). The median CLO concentration in pooled seeds collected within 24 h post-sowing was 254.5 μg/g (inter-quartile range (IQR) = 173.6; n = 27), compared to 90.3 μg/g (IQR = 154.7; n = 33) in seeds collected 2–7 days post-sowing and 48.2 μg/g (IQR = 83.6; n = 16) in those collected 7–14 days post-sowing. There was also a significant negative association between CLO residue in seed samples and cumulative rainfall at each field (Table 2). The loss of CLO from seeds sampled at the earliest compared to the latest day post-sowing (at any one field, in either year; n = 20) yielded an average loss of 13% of remaining residue per mm of rain.

CLO residue measured in individual seeds collected within 24 h of sowing varied around the target application rate of 500 μg/g (calculated from the Redigo Deter® product label, which states that 200 mL (containing 50 g of CLO) should be applied to 100 kg of seed (Bayer Crop Science UK, 2019)), with CLO concentrations ranging between 104.6 and 606.9 μg/g per seed (Fig. S1). The mean (±SE) residue in individual seeds collected within 24 h of sowing was 278.3 ± 19.4 μg/g and the coefficients of variation for groups of individual seeds collected at each of the four fields within this time period ranged from 22 to 39%. Individual seeds collected 24 h post sowing contained on average 55.6% of the target application of CLO.

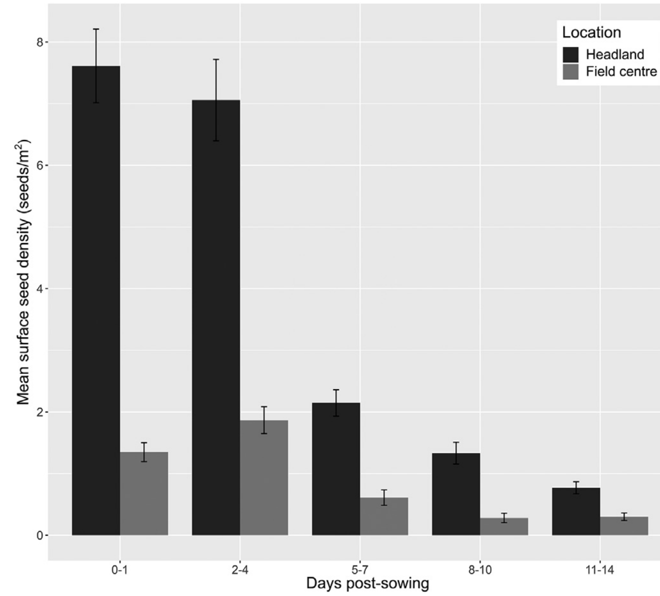

Fig. 1. Surface seed densities between day 0 (sowing date) and 14-days post-sowing. Mean surface seed density per m^2 was calculated across all farms (n) for days 0–1 (n = 24), 3 ± 1 (n = 20), 6 ± 1 (n = 25), 9 ± 1 (n = 20) and 12 ± 1 (and data from one farm collected on day 14; n = 24) post-sowing, with standard error bars. Data are shown separately for headland and field centre.

Table 2
Summary of generalised linear mixed model outputs for surface seed density and seed residue data collected from East Anglia.

Model	Model output			
	Disp	Est	SE	p-Value
Surface seed density ~ location within field + number of days post sowing + year				
Location within field	0.09	0.33	0.03	<**0.001**
Number of days post-sowing		−0.14	0.01	<**0.001**
Year		−0.12	0.07	0.076
CLO residue in seeds ~ number of days post sowing + cumulative rainfall				
Number of days post sowing	0.59	−0.10	0.06	0.088
Cumulative rainfall		−0.14	0.01	<**0.001**

Disp: model dispersion; Est: model estimate; SE: standard error; CLO: clothianidin.
Values in bold are indicate statistical significance ($p < 0.05$).

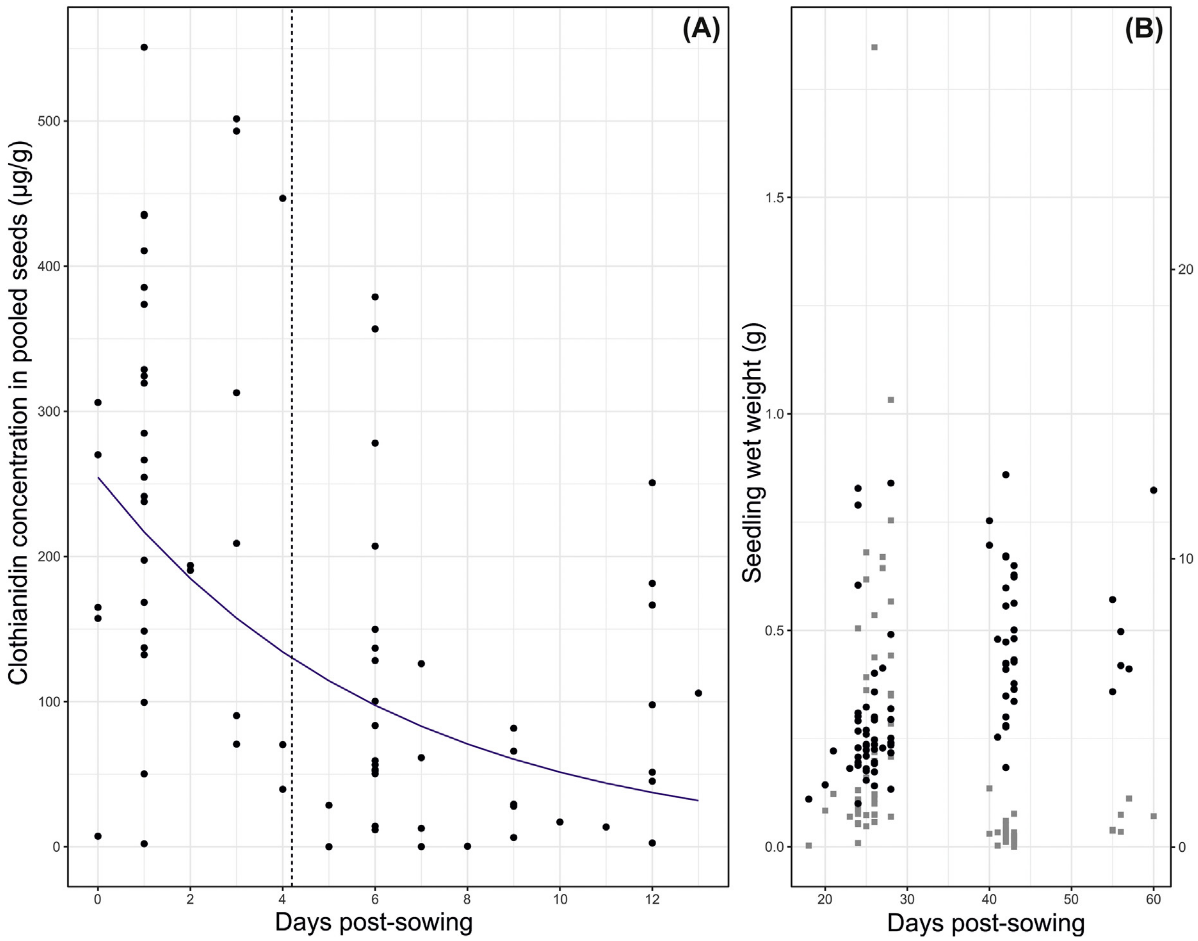

Fig. 2. A) Concentration of clothianidin (CLO) in pooled seed samples collected between 0 and 14 days post-sowing. Each data point represents a pooled sample, or the mean taken from a group of individual samples from the same site and on the same day. Blue line: curve describing dissipation of residues on seeds over time. Dashed line: dissipation half-life (4.2 days) calculated using all sample values. B) Wet weight of single seedlings and concentration of CLO in seedling samples between days 18 and 60 post-sowing. Grey squares represent the concentration of CLO in each seedling sample (individual and pooled). Black circles represent the weight of each seedling; data points are either the weight of an individual seedling or an estimate of individual seedling weight calculated by dividing the weight of a pooled sample by the number of seedlings in that pool. All samples with a concentration >15 µg/g are seedlings that were analysed individually; one outlier was removed from these data (seedling weight = 0.17 g, CLO concentration = 104.5 µg/g, collected 28 days post-sowing). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2.2. Seedlings

The median residue in pooled seedling samples was 1.1 µg/g (IQR: 1.4; n = 34), which was 122-fold lower than the median residue measured in pooled seed samples. Residue also varied greatly in pooled seedling samples across the study period (0.003–15.8 µg/g). The median residue in pooled seedlings collected 2–4 weeks post-sowing was 1.8 µg/g (n = 17), compared to 0.5 µg/g (n = 17) for those collected 5–13 weeks post-sowing (representative of the two seedling growth stages sampled). The concentration of CLO decreased significantly with increasing seedling mass (Spearman's rank correlation $r_s = -0.305$, p = 0.003; Fig. 2B). As with seeds, residue in seedlings did not differ significantly between fields (Kruskal-Wallis $\chi^2_{17} = 18.5$, p = 0.356).

The concentration of CLO measured in individual seedlings ranged between 0.1 and 104.5 µg/g (mean: 4.8 ± 1.8 µg/g), with coefficients of variation for groups of individual seedlings from each field ranging between 124 and 198%. On average seedlings contained 5.9% of the CLO residue recorded in seed samples collected 0–2 days post-sowing (based on the mass of CLO per seed or seedling across pooled and individual samples).

3.3. Bird survey & camera trap data

A total of 65 bird species were recorded in fields sown with treated seed during the surveys undertaken in 2015 and 2016 (Table S3). Songbirds made up the largest proportion of species observed in treated fields throughout the study period, whilst gulls accounted for several of the larger numbers of birds observed (Fig. S2A). Starlings *Sturnus vulgaris* were the most frequently observed songbird, accounting for 48% of all observations, followed by finch species (26%), comprised of large flocks of linnet *Linaria cannabina* (Fig. S2B).

A significant positive association was found between mean surface seed density (calculated for each field, at each survey visit) and bird abundance (recorded on the same field and survey visit) for those species where agricultural crop seed was recognised as being 'present' in the diet, but no association was found for those species where crop seed was deemed 'absent' from the diet (Table 3; Fig. S3A) (Cramp, 1985). When data were analysed for taxonomic guilds that are known to consume agricultural seed, surface seed densities were found to be positively associated with the number of 'other passerines' (starling observations accounted for 79% of data points in this guild) and buntings (comprised of yellowhammer *Emberiza citrinella* and reed bunting *Emberiza schoeniclus* observations; Fig. S3). Gamebirds exhibited a weaker positive association (p = 0.083; Fig. S3), but no significant association was detected for crows, finches, gulls or columbids (Table 3).

Fifteen bird species were observed consuming treated-seed at seed piles (Table 4). The maximum time spent and number of seeds consumed at a seed cluster during any single visit was

Table 3
Summary of generalised linear mixed model outputs for avian data. Bird abundance (up to 14 days post-sowing for specific taxonomic guilds) was modelled as a function of surface seed densities.

Model	N species	Model output			
		Disp	Est	SE	p-Value
Bird abundance ~ seed density					
Species with agricultural crop seed absent in diet	37	1.09	0.07	0.08	0.418
Species with agricultural crop seed present in diet	34	1.27	0.26	0.07	<**0.001**
Bird abundance (species with agricultural seed present in diet, split by taxonomic guild) ~ seed density					
Buntings (*Emberizidae*)	2	0.41	0.38	0.16	**0.018**
Crows (*Corvidae*)	5	0.68	0.20	0.14	0.148
Finches (*Fringillidae*)	3	0.45	0.17	0.22	0.447
Gamebirds (*Phasianidae*)	3	0.53	0.25	0.14	**0.083**
Gulls (*Laridae*)	5	0.38	0.16	0.20	0.408
Other passerines (*Alaudidae*, *Passeridae*, *Prunellidae*, *Sturnidae*)[a]	4	0.58	0.43	0.18	**0.015**
Pigeons & doves (*Columbidae*)	4	0.55	0.23	0.17	0.194
Thrushes (*Turdidae*)	1	0.59	0.18	0.14	0.193

See Table S3 for a full list of species included in each 'taxonomic guild' used for bird abundance data.
[a] Shorelark *Eremophila alpestris* excluded from the model (only one individual recorded throughout survey period). Starlings *Sturnus vulgaris* made up 79% of observations in this group. Est: model estimate; Disp: model dispersion; N: number of; obs: observations; SE: standard error for model estimate.

11 min and 15 seeds (woodpigeon *Columba palumbus*; Table S4). Individual birds at seed clusters were found to consume 1.4–65.2% and <0.1–3.2% of the sub-lethal and lethal threshold for CLO, respectively, per visit (based on the mean and maximum number of seeds consumed and the mean concentration of CLO detected on seeds in the present study – 0.016 mg/seed; Table 4); it must be noted that these toxicity values can only be applied directly to the tested species and that other species may be more or less sensitive. In general, smaller species (<30 g body weight) were found to ingest a larger proportion of the amount of compound required to reach either toxicity threshold compared to larger species (>30 g body weight; Table 4).

3.4. Clothianidin residue: avian plasma samples

Significantly more avian plasma samples tested positive in the post-sowing group (36/71, ~51%), compared to the pre-sowing control group (4/36, ~11%; Fisher's exact: OR = 8.0, CI = 34.7, $p < 0.001$). Samples were available from ten species post-sowing and nine species pre-sowing, of which nine and two species tested positive for CLO, respectively. Greenfinch *Chloris chloris* was the only species to test negative in the post-sowing group, whereas blackbird *Turdus merula* and starling were the only species to test positive in the pre-sowing group (3/5 and 1/1 birds tested, respectively; Table 5). All four birds that tested positive pre-sowing were female, whereas males and females were equally represented in samples collected post-sowing. Concentrations of clothianidin in all positive samples ranged between 0.5 and 69,300 ng/mL, with a median value of 12.0 ng/mL (n = 40; Table 5). The median CLO concentration in positive samples collected pre-sowing was 3.6 ng/mL (n = 4), whereas the median in post-sowing samples was 12.5 ng/mL (n = 36).

There was a significant difference between the concentration of CLO found in avian plasma samples collected from sites adjacent to different fields post-sowing (Kruskal-Wallis $\chi^2_5 = 17.4$, p = 0.003; Supplementary Note 2). However, there was no significant difference in the concentration of CLO recorded post-sowing between species (with five or more positive samples; Kruskal-Wallis $\chi^2_4 = 2.4$, p = 0.662; Table 5). For species where measurements of CLO concentration in plasma were available, four were observed consuming treated seeds at seed clusters (dunnock, robin *Erithacus rubecula*, house sparrow *Passer domesticus* and chaffinch *Fringilla coelebs*) and five were observed in treated fields (yellowhammer, blackbird, reed bunting, goldfinch *Carduelis carduelis* and starling); all tested positive for CLO (Table S3). Tree sparrow *Passer montanus* was the only species to test positive for CTD that was not observed in treated fields in East Anglia, whereas greenfinch was observed in treated fields in East Anglia, but did not test positive for CLO (only one sample was obtained for analysis). Two individuals with the highest CLO concentrations in plasma samples for their species (yellowhammer and tree sparrow; 69,300 and 4880 ng CLO/mL, respectively) exhibited intoxication symptoms at sampling (fluffed up appearance, sluggish movement) and had red dye around their bills. Both these individuals, in addition to a third (chaffinch *Fringilla coelebs*; 5 ng CLO/mL in plasma) also had red faeces.

Table 4
Summary of camera trap data for bird species observed consuming treated seed at seed clusters. Data are ordered by the maximum proportion (%) of the toxicity thresholds (for CLO) that each species consumed.

English name	Latin	Average species weight	Total individuals	Seeds eaten per individual, per event			% of CLO toxicity threshold reached (mean[a])		% of CLO toxicity threshold reached (max[a])	
		(*g*)	(*n*)	*Mean*	*SE*	*Max*	LD_{50}	*NOAEL*	LD_{50}	*NOAEL*
Woodpigeon	*Columba palumbus*	507	115	9.37	1.63	152	0.2	4.0	3.2	65.2
Dunnock	*Prunella modularis*	21	4	2.25	0.48	3	1.1	23.3	1.5	31.1
Chaffinch	*Fringilla coelebs*	22	14	1.36	0.17	3	0.7	13.4	1.5	29.6
House sparrow	*Passer domesticus*	27	16	1.81	0.16	3	0.7	14.6	1.2	24.2
Feral pigeon	*Columba livia domestica*	360	4	19.25	6.88	37	0.6	11.6	1.1	22.3
Magpie	*Pica pica*	213	34	4.29	0.54	13	0.2	4.4	0.7	13.3
Red-legged partridge	*Alectoris rufa*	530	48	6.42	0.78	28	0.1	2.6	0.6	11.5
Robin	*Erithacus rubecula*	19	4	1.00	0.00	1	0.6	11.4	0.6	11.4
Jay	*Garrulus glandarius*	167	1	7.00	n/a	7	0.4	9.1	0.4	9.1
Grey partridge	*Perdix perdix*	490	31	5.55	0.88	20	0.1	2.5	0.4	8.9
Carrion crow	*Corvus corone*	509	61	5.05	0.62	19	0.1	2.2	0.4	8.1
Rook	*Corvus frugilegus*	452	8	3.88	1.38	13	0.1	1.9	0.3	6.3
Pheasant	*Phasianus colchicus*	1200	21	6.95	1.42	22	0.1	1.3	0.2	4.0
Stock dove	*Columba oenas*	326	1	5.00	n/a	5	0.2	3.3	0.2	3.3
Jackdaw	*Corvus monedula*	232	2	1.50	0.50	2	0.1	1.4	0.1	1.9

LD_{50}: median lethal dose; NOAEL: no-observed-adverse-effect level; SE: standard error of the mean.
[a] Calculated using the mean or maximum number of seeds consumed per visit for each species, an estimated concentration of 0.016 mg of CLO per seed (equal to the average mass of CLO per individual seed in this study). Endpoint values for NOAEL and LD_{50} were obtained from Mineau and Palmer (2013), for a 15 g bird at the 5% tail of sensitivity, which were moderated by the average weight for each species (obtained from the BTO (Robinson, 2005)). NOAEL in this instance refers to reproductive effects only (Mineau and Palmer, 2013).

Table 5
Summary of the prevalence of clothianidin (CLO) in avian samples collected post sowing and the concentrations of the compound measured in individual plasma samples collected from each species. Data are ordered by maximum concentration measured in any one individual bird from one species (from highest to lowest). CLO prevalence post-sowing is calculated to the nearest 1%.

Species		Number of samples pre-sowing			Number of samples post-sowing			CLO prevalence post-sowing	Residue in all positive samples (ng/mL)			
		Total	*ND*	*POS*	*Total*	*ND*	*POS*	*%*	*Minimum*	*Maximum*	*Median*	*IQR*
Yellowhammer	*Emberiza citrinella*	0	0	0	10	3	7	70	2.0	69,300	29.4	4530
House sparrow	*Passer domesticus*	2	2	0	5	3	2	40	6740	7500	7120	380
Tree sparrow[a]	*Passer montanus*	4	4	0	9	3	6	60	3.3	4880	22.5	37.2
Chaffinch	*Fringilla coelebs*	8	8	0	9	2	7	78	0.6	3520	29.3	1000
Dunnock	*Prunella modularis*	8	8	0	15	10	5	30	0.5	444	3.7	54.3
Blackbird	*Turdus merula*	5	2	3	7	2	5	71	2.4	127	9.4	8.0
Reed bunting	*Emberiza schoeniclus*	0	0	0	6	5	1	15	3.0	3.0	3.0	n/a
Starling	*Sturnus vulgaris*	1	0	1	0	0	0	0	2.0	2.0	2.0	n/a
Robin	*Erithacus rubecula*	1	1	0	1	0	1	100	1.7	1.7	1.7	0.0
Goldfinch	*Carduelis carduelis*	6	6	0	8	6	2	25	0.8	1.4	1.1	0.3
Greenfinch	*Chloris chloris*	1	1	0	1	1	0	0	n/a	n/a	n/a	n/a

ND: non-detect for CLO; POS: tested positive for CLO; IQR: inter-quartile range; n/a: not applicable.
[a] Tree sparrow was not observed in treated fields in East Anglia (Table S3) due to their restricted range (see https://app.bto.org/mapstore/specieschooser.jsp).

4. Discussion

Results from this study collectively confirmed that 21 species of farmland bird were exposed to CLO, thus documenting exposure to an avian community over a typical period of sowing CLO-treated winter cereals. Exposure was identified via direct observations of CLO ingestion via treated seed (15 species) and/or the presence of CLO residue in plasma (10 species), in approximately one third of all species observed in CLO-treated fields. The median concentration of CLO recorded in plasma samples here was larger than any NN residue reported in an avian species to date (Taliansky-Chamudis et al., 2017), except for poisoning incidents (Millot et al., 2017). The study took place in autumn when alternative sources of food for farmland birds are relatively abundant; it is thus possible that exposure would be greater during sowing of spring cereals when food is more scarce (Siriwardena et al., 2008). This study provides evidence that seed treatments are a source of CLO exposure in wild birds, and identifies multiple factors that may affect patterns of exposure observed in the field.

According to application instructions provided by the manufacturer, treated seeds are required to be buried at a depth of 4 cm and are to be reincorporated into the soil if left on the soil surface after drilling (Bayer Crop Science UK, 2019). Here, CLO-treated seeds and seed clusters were available on the soil surface at the majority of fields surveyed, which is in accordance with previous research that identified high prevalence of wheat seed at the soil surface compared to other crop types (de Snoo and Luttik, 2004; Roy et al., 2019). Thus there is a gap between the regulatory expectation that treated seeds can be entirely buried, and the agricultural reality that complete removal of treated seed from the soil surface cannot be achieved in practice. The current study found that treated seed was present on the soil surface in almost all the fields surveyed across both years, whilst seed clusters were found at 79% of fields sampled. However, the number of seeds on the soil surface differed significantly between fields, suggesting non-uniformity across drilling practices; this variability has previously been attributed to differences in soil type or farm machinery (de Snoo and Luttik, 2004; Roy et al., 2019). Surface seed density was also higher at the headlands compared to field centres as found in previous studies (de Snoo and Luttik, 2004; McGee et al., 2018; Roy et al., 2019; Lopez-Antia et al., 2016), and may be indicative of differences in localised efficiency of farm machinery at burying seeds at the prescribed depth (Bayer Crop Science UK, 2019).

The median concentration of CLO on pooled samples of treated seed collected from the soil surface within the first 24 h after sowing was 49% lower than the target application rate (as prescribed by product labels), and did not differ significantly between fields. However, there was high intra-field variability in CLO concentrations on seeds collected and analysed individually from four fields. These results tally with a similar study that also found variable concentrations of NNs that were below the application rate on soybean and corn seeds (Roy et al., 2019) and may partially be attributable to seeds taking in moisture once exposed to the external environment. The dissipation half-life for CLO on treated seed was 4.2 days when calculated across our full sample set, which is somewhat longer than the 2.0–2.3 days previously reported for CLO on maize and soybean seed left on the soil surface in Minnesota, USA (Roy et al., 2019). Dissipation is likely to proceed primarily via photodegradation (e.g. Li et al. (2018b) reported a half-life of 13 h for pure CLO exposed to natural sunlight) coupled with some wash off by rainfall.

Comparatively, the amount of residue measured in seedlings was considerably smaller than that in seeds (on average seedlings contained only 5.9% of the CLO measured in treated seeds), which tallies with previous studies that have found between 1 and 15% of residue in treated seeds is taken up by the seedling (Prosser and Hart, 2005; Alford and Krupke, 2017); the measured value in the current study is three times smaller than the European regulatory default for risk assessment purposes (European Food Safety Authority, 2010). CLO concentrations were negatively associated with the wet weight of individual seedlings, presumably reflecting growth dilution of residues (see also (Balfour et al., 2016)) as seedlings developed. Similar patterns of CLO concentrations were found in seedling samples compared to seeds, which also exhibited low inter-field variability in pooled samples, but high intra-field variability in individual samples. These results highlight the potential for large variability in exposure arising from the consumption of either seeds or seedlings. Data here suggest that treated seedlings are a smaller source of exposure to farmland birds when compared with treated seeds; however, seedlings may be more widely available in the agricultural landscape as this is independent of any risk mitigation measures, and will be a source of exposure to some herbivorous species that do not consume seeds.

During the period 0–13 weeks post-sowing, 66 species of bird were recorded in fields in East Anglia and Lincolnshire that were sown with CLO-treated seeds. Of these, exposure was confirmed in some individuals from 32% of all species recorded (Table S3). The species exposed were not restricted to any one taxonomic group: plasma samples tested positive in species of sparrow, bunting, finch and thrush, whilst species of columbid, galliforme, corvid and passerine were observed consuming treated seed at spilt seed clusters. Observations relating to galliformes and columbids are consistent with previous observations of NN poisonings during autumn months and the detection of NN residues in samples of liver and eggs collected from quail, partridge and pigeon (Bro et al., 2016; Millot et al., 2017; Ertl et al., 2018; Turaga et al., 2016). Furthermore, exposure was confirmed here for a similar species composition to that in Roy et al. (2019), and 12 of the 30 species observed consuming seeds treated with pesticides in a previous study conducted in Spain (Lopez-Antia et al., 2016). This included multiple sparrow species, such as house sparrow, which have also been reported to be extensively exposed to NNs across the Swiss plateau (Humann-Guilleminot et al., 2019a). Overall exposure was not limited to any specific species ecology or taxonomy, other than that the majority of species exposed are known to have cereal grain in their diet (Table S3) (Cramp, 1985).

The prevalence of CLO residues in plasma samples collected post-sowing (~50%) was broadly similar to that reported previously; of the three other studies that have measured NN residue in plasma samples collected from wild birds, positive samples accounted for 3% ($n = 30$ bird of prey samples), 60% ($n = 10$ bird of prey samples) and 80% ($n = 36$ passerine samples) of the total sample size (Byholm et al., 2018; Taliansky-Chamudis et al., 2017; Hao et al., 2018). Comparatively, Humann-Guilleminot et al. reported 100% prevalence of NN residue in 146 pooled house sparrow feather samples (each pool contained one feather from three individuals) (Humann-Guilleminot et al., 2019a). When comparing these data, differences in sample type, time of sampling (in relation to exposure) and the ecology of the species investigated are all likely to explain the observed variation between studies. Firstly, pooled samples may inflate the overall exposure prevalence compared to samples analysed from single birds. Furthermore, NN in feathers may have been laid down over a period of several days or weeks during moult, whereas NN residue is known to exit the blood stream 6–8 h after the compound is ingested (Hao et al., 2018; Bean et al., 2019). Therefore blood residues are more likely to provide a snap-shot of exposure whilst feather residues may reflect aggregated exposure over a longer period. Secondly, compared to passerines, it is much less likely that birds of prey will experience primary exposure via the direct ingestion of treated seed. This will predispose passerines to higher levels and frequencies of exposure than predatory species.

Notably, the median and maximum concentration of CLO in plasma recorded in the present study exceeded any previous records of NN residue in avian plasma. To date, 3.28 ng imidacloprid/mL was the highest NN concentration reported for a bird of prey plasma sample (obtained from Eurasian eagle owl *Bubo bubo* (Taliansky-Chamudis et al., 2017)) and 0.17 ng imidacloprid/mL the highest NN concentration in a passerine plasma sample (obtained from a white-crowned house sparrow *Zonotrichia leucophrys* (Hao et al., 2018)). Here we recorded a median concentration across all positive samples of 12 ng CLO/mL, whilst the maximum concentration recorded (in one yellowhammer) was 69,300 ng CLO/mL. As this is the first study to measure residue of a NN in plasma samples collected directly post-sowing (compared to those conducted outside of the sowing season), it is possible that these data are not unusual during this time period and may be representative of a period of peak exposure as a result of the increased availability of treated seed (Supplementary Note 2).

Surveys in East Anglia confirmed that surface seed densities were significantly associated with bird abundance in treated fields for species groups such as buntings and passerines, as well as gamebirds. These abundance observations tally with those species that were seen to have the highest concentrations of CLO in plasma samples (such as yellowhammer and tree sparrow), as well as multiple gamebird species that were observed consuming treated seeds at seed clusters. Starling made up the largest proportion of birds observed in treated fields; only one plasma sample was available for this generalist species, but it yielded a positive residue of CLO. Interestingly, starling and blackbird were the only two species to test positive for CLO pre-sowing, both of which are migratory and highly dispersive in autumn (Cramp, 1985) and are likely to have had access to sites outside of those sampled where drilling had already taken place. Also of note is that species such as goldfinch, reed bunting and blackbird are not typically known to consume cereal seed (Holland et al., 2006), so alternative exposure pathways aside from ingestion of treated seed should be considered. Lopez-Antia et al. (2016) reported imidacloprid exposure in partridges that was not associated with seed treatments, and previous research has evidenced off-site contamination of soil, water and wild plants with CLO originating from seed treatments (Botias et al., 2016; Humann-Guilleminot et al., 2019b). This hypothesis is further supported by literature suggesting that NNs leach from treated seeds over time in response to precipitation (Radolinski et al., 2019; Radolinski et al., 2018).

Although this study confirms that birds are exposed to CLO, what remains less clear is the impact that this level of exposure is likely to have on avian fitness and health in the wild. When examining CLO toxicity thresholds in the context of the number of seeds consumed at seed clusters, one wood pigeon was found to ingest sufficient seed to reach 65% of the generic NOAEL threshold for reproductive effects, whilst smaller species (<30 g) were found to ingest 11 to 31% of the compound required to reach the reproductive NOAEL threshold per feeding event. These estimations are constructed based on single visits, so could be under-estimating exposure when considering availability of treated seed in the broader landscape. Notably, the two individuals (one tree sparrow and one yellowhammer) that had the highest CLO plasma concentrations for their species (4880 and 69,300 ng/mL, respectively) exhibited intoxication symptoms at time of capture, which were similar to those described in imidacloprid-dosed eared doves (Addy-Orduna et al., 2018). These individuals also had red dye around the bill and red faeces, indicating recent ingestion of CLO-coated seeds, as has similarly been reported in NN poisoning incidents (Millot et al., 2017). It is likely that the concentrations of CLO in the blood stream breached a toxicity threshold for these individuals, although toxicological data for CLO are not available for these particular species to confirm this.

5. Conclusion

Results here provide clear evidence that a variety of farmland birds are subject to widespread CLO exposure following normal agricultural sowing of treated winter cereal seed. CLO exposure was confirmed in 32% of species observed and 50% of individuals sampled in treated fields post-sowing, with levels of exposure to CLO among the highest recorded for wild birds to date. The widespread availability of winter wheat seeds at the soil surface was identified as a primary source of exposure. Factors such as the variation in compound application to seed, rainfall patterns after sowing, and differences in drilling efficiency between farms are likely to have contributed to temporal and spatial variability in exposure. Whilst outdoor use of imidacloprid, thiamethoxam and CLO has been banned in the EU, the information reported here is likely to have implications for multiple bird species where NNs are in use and may help to inform any future policy decisions related to this group of insecticides. In addition, these data are pertinent to future risk assessments through identifying consumption of treated winter cereal seed as a source of exposure, and thus risk, to a wide range of species of farmland bird.

Ethics statement

All works were approved by the Animal Welfare and Ethical Review Body at the University of York. The Home Office reviewed and approved the licence to take blood samples from species in the study (licence number: P3AF8F232).

CRediT authorship contribution statement

Rosie J. Lennon: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Will J. Peach:** Conceptualization, Data curation, Methodology, Resources, Supervision, Writing - review & editing. **Jenny C. Dunn:** Investigation, Methodology, Resources, Supervision, Writing - review & editing. **Richard F. Shore:** Methodology, Supervision, Writing - review & editing. **M. Glória Pereira:** Formal analysis, Resources, Supervision, Writing - review & editing. **Darren Sleep:** Formal analysis. **Steve Dodd:** Investigation. **Christopher J. Wheatley:** Data curation. **Kathryn E. Arnold:** Funding acquisition, Resources, Supervision. **Colin D. Brown:** Funding acquisition, Methodology, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.138056.

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