Field evaluation of the potential for avian exposure to clothianidin following the planting of clothianidin-treated corn seed

Sean McGee Corresp., [1] , **Melissa Whitfield-Aslund** [2] , **Daiana Duca** [2] , **Nicole Kopysh** [2] , **Tereza Dan** [2] , **Loren Knopper** [2] , **Larry Brewer** [3]

[1] Bayer CropScience, LP, United States
[2] Stantec Consulting Ltd, Canada
[3] Compliance Services International, United States

Corresponding Author: Sean McGee
Email address: sean.mcgee@bayer.com

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Sean McGee[1], Melissa Whitfield Aslund[2], Daiana Duca[2], Nicole Kopysh[2], Tereza Dan[2], Loren Knopper[2], Larry Brewer[3]

[1] Bayer CropScience, LP
[2] Stantec Consulting Ltd.
[3] Compliance Services International

Corresponding Author: Sean McGee[1]
Corresponding Author Email: sean.mcgee@bayer.com

ABSTRACT

This objective of this study was to quantify consumption of clothianidin-treated corn seed by birds following standard planting practices. Post-planting seed counts on 21 fields in southwestern Ontario, Canada, demonstrated that a small proportion of total sown treated seeds remained on the soil surface immediately post-planting (i.e. mean 0.3 ±0.2% of the total sown seeds). Behavior monitoring of individual birds and 24-hr remote video surveillance were deployed to investigate how much of the treated seed remaining on the soil surface was consumed by birds. Spotting scopes were used to monitor the full duration of the field visits of 596 individual birds during morning hours for three consecutive days after planting on each of the 21 fields. Only two birds were observed consuming treated seeds (one seed each) and three birds consumed seeds for which the treatment status could not be visually confirmed. Similarly, in > 1,380 hours of continuous video monitoring of field locations with the highest likelihood of avian exposure (where multiple treated seeds remained clustered on the soil surface), no birds were observed eating treated seed. This study provides field verification on two factors that determine exposure: 1) standard sowing practices in Ontario are effective at burying treated seeds such that the count of seeds on the soil surface after planting is low, and 2) foraging birds monitored on these fields consumed very few of the clothianidin-treated corn seeds remaining on the soil surface after planting.

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INTRODUCTION

Clothianidin and other systemic neonicotinoid insecticides such as imidacloprid, thiamethoxam and dinotefuran are readily taken up by and circulated within plants, where they provide the plant with a period of systemic protection against biting and sucking pests (e.g., aphids, whiteflies, thrips, leafhoppers, scales, and leaf miners; Bonmatin et al., 2015; EPA 2017a). This attribute allows neonicotinoids to be applied as seed treatments for a variety of crops, including corn, with a lower environmental loading of chemical than would result from soil-applications or foliar sprays. Therefore, the use of seed treatments can reduce the pesticide input per unit of area while protecting the seeds and young plants from damaging pests.

Because treated seeds represent a potential food source for granivorous birds that forage on agricultural fields, concerns have been raised regarding the potential for direct toxic effects to birds due to consumption of treated seed (Lopez-Antia et al., 2015; Millot et al., 2017; EPA 2017a). However, there have not been any confirmed avian mortalities associated with registered clothianidin uses (EPA 2017a). This is in part due to the low dietary toxicity of clothianidin (LC50 >5230 mg clothianidin/kg diet) as well as planting practices and bird behaviors that may limit exposure of birds to clothianidin-treated corn seeds under realistic field conditions (EPA 2017a).

For example, corn seeds are planted at depth (typically 38 – 51 mm below the soil surface to maximize root development) and are therefore unlikely to be available on the surface for consumption by birds. In their interim guidance on how to refine risk assessments for pesticide treated seeds, EPA (2016) suggested that an incorporation rate of 99% should be assumed for seeds (like corn) that are planted underground with an in-furrow planter or seed drill (i.e., only 1% of seeds should be considered available for bird consumption). Additionally, the directions on the product label for clothianidin-treated corn-seeds (e.g., Poncho® 600 [label]; Bayer CropScience) mandate that spilled or exposed seeds must be incorporated into the soil or otherwise cleaned up from the soil surface. Therefore, standard agronomic practices are expected to limit the number of treated corn seeds available on the surface after planting.

Additionally, previous studies have found that if undetected treated seeds do remain on the surface after planting, birds may avoid consuming them due to their altered appearance (i.e., color, texture or size) and/or may learn to avoid treated seeds after a first experience of post-ingestion distress (de Leeuw et al., 1995; Hartley et al., 2000; OECD, 1996; Avery et al., 1994; Lopez-Antia et al., 2014; Millot et al., 2017). For clothianidin-treated corn seeds, seed acceptance studies sponsored by Bayer CropScience have demonstrated significant avoidance of these seeds by both the domestic pigeon (*Columba livia*) and common pheasant (*Phasianus colchicus*), even under fasted, no choice conditions (Unpublished studies; see Supplemental Table S1). Other factors that affect treated seed consumption and resulting pesticide exposure include seed size and seed handling behavior. For treated seeds that have husks (e.g., millet, rice, sunflower and sorghum), seed handling behavior (e.g., removing and discarding the seed husks to access the seed kernel) can substantially reduce exposure because the active ingredient is mainly on the husk of the seed (Avery et al., 1997). Corn seeds do not have a shell or husk; however, with an approximate mass of 225 mg, they are too big for small (e.g.., 20 g) to medium (e.g., 100g) sized passerine birds to consume whole (EPA, 2017a, Benkman and Pulliam, 1988; Diaz, 1990; Diaz, 1994). Instead, these birds may work to crack the kernel and eat only the inner endosperm of the corn seed, which will also reduce exposure since the clothianidin treatment is found on the outer surface of the corn seed. Therefore, undetected seeds remaining on the surface may be avoided or only partially consumed.

To investigate how planting practices and bird foraging behavior may affect potential exposure of birds to clothianidin-treated corn seeds under normal, label compliant, agronomic practices, this study was designed to 1) evaluate how many clothianidin-treated corn seeds remain available on the soil surface of monitored field plots after planting, and 2) observe how frequently (if at all) these remaining seeds were consumed by birds. Twenty-one corn fields in southwestern Ontario, Canada, were observed in the first three days after planting to determine seed sowing efficiency, frequency and duration of wild bird visits, and foraging behavior and food consumption on the fields by wild birds. Birds were also monitored for acute clinical symptoms of toxicity or mortality while they were present on the monitored field plots.

Where possible, study plot conditions were selected to maximize seed availability and attractiveness to birds and thereby capture realistic upper-limits of exposure. For example, monitoring was carried out in the first three days after planting, when clothianidin residues are likely to be highest (clothianidin residues on seeds are known to decline rapidly due to weathering) and seeds remain available (i.e., prior to germination). Additionally, avian monitoring focused on areas where seed availability was highest (e.g., turn rows and edge rows) and in field plots adjacent to suitable avian habitat (i.e., more likely to attract birds).

METHODS

This field study was conducted in accordance with US EPA Good Laboratory Practice Standards set forth under the Federal Insecticide, Fungicide, and Rodenticide Act (40 CFR, Part 160).

Site Selection

Potential field cooperators from Southern Ontario were identified from a list of growers that had purchased clothianidin-treated corn seed (Poncho®, Bayer CropScience) at the highest available treatment rate (nominal 62.5 mg clothianidin/50 seeds, equivalent to nominal 1.25 mg clothianidin/seed). From this list, cooperator fields that were to be planted with clothianidin-treated seed were surveyed and selected for inclusion in the study based on grower agreement and a minimum distance of 800 meters separating fields. To maximize the potential for bird observation, only fields adjacent to suitable avian habitat (i.e., non-crop, non-pasture) were selected (i.e., where birds are more likely to be present). The final selected sites included 10 fields in the vicinity of Guelph, Ontario and 11 fields in the vicinity of London, Ontario (see Supplemental Fig. S1).

Analytical Verification of Clothianidin Concentration on Seeds

A single commercial seed company delivered clothianidin commercially treated corn seeds directly to the cooperating growers and the seed treatment rate, lot number and quantity delivered to each cooperating grower was verified. Samples of clothianidin treated corn seeds were collected by study team members

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from five different fields from seed bags just before planting, or from the seed hopper during planting, and shipped to Smithers Viscient in Wareham, MA for verification of the amount of test substance per seed. A subsample of 50 seeds from each of the sampled fields was analyzed for clothianidin by high performance liquid chromatography with ultraviolet detection (HPLC/UV). The test substance was extracted from the seeds using acetonitrile-water (1:1 vol/vol) as the extraction solvent. HPLC was performed at 40˚C using a mobile phase of (A) 0.1% acetic acid in water and (B) 100 % acetonitrile. The flow rate was 1.5 mL/ min, with an injection volume of 5 µL and the eluent was monitored at 294 nm. The method was verified using an analytical standard (clothianidin) of 99.9% purity diluted in acetonitrile-water (1:1 vol/vol). Quality control (QC) samples spiked with known concentrations of clothianidin covering a specified range (low, mid and high) were used to determine the accuracy of the method. The acceptable recovery limit for QC samples was set at 80-120%. The limit of quantification (LOQ) was 0.14 mg/mL.

Planting of Treated Seeds

Planting was conducted in May to June by each grower using their personal planting equipment at planting rates that are typical for the region (i.e., 69,200 – 88,900 corn seeds per hectare; Ontario Ministry of Agriculture, Food and Rural Affairs, 2017). Following typical “plant-to-moisture” practices for seed depth, planting depths were between 2.5 to 5 cm and varied based on the time since last rainfall and moisture retention of the soil. Planting practices were left up to the individual growers without interference to assure a realistic corn-planting scenario.

Post-Planting Counts of Seeds on the Soil Surface

The commercially available clothianidin-treated corn seeds evaluated in this study were purple due to treatment with an approved purple dye. Therefore, they were readily visually distinguishable from untreated (yellow) seeds. Immediately following planting, transects were established to count the number of corn seeds remaining on the soil surface, either left by the planter or because of an accidental spill event. Corn furrows were numbered and marked starting from one edge of each field. Thirty to sixty rows were selected to establish transects for counting seeds remaining on the soil surface (Table 1). Based on the seed row length, row spacing, and total number of rows included in each transect, the size of the transects that were directly monitored for corn seed counts ranged from 0.53 to 0.85 hectares (Table 1). Given that the transects traversed across field centers, near-edge areas, and turn rows, the seed counts (per hectare) observed in these transects can be considered representative of the entire field.

To perform the seed count, observers walked just to the side of the marked seed furrows and counted and recorded treated corn seeds visible on the soil surface in a swath that extended half the distance of the row spacing on both sides of the furrow. Mechanical tally recorders were used to ensure the count was not lost during the process. During the seed counts carried out immediately after planting (i.e., Day 0), observers marked seed spills or areas of multiple seeds remaining on the soil surface with small plastic plant tags, including a tag number and the number of seeds visible for a given spill. After three days, the corn seeds at each tagged location were recounted to determine the number of seeds that were missing.

Characterization of Avian Species Visiting Study Fields and Observations of Avian Behavior on Treated Fields

One completely observable subplot on each field was established and marked with flags prior to planting. In this study, it was determined that an individual observer could effectively monitor birds and bird behaviors across an area approximately 300 m^2. Therefore, the subplots were limited in size to $\leq$ 300 m x 300 m at each field. The size and shape of each subplot differed slightly due to differences in topography in each field. However, in all cases, the subplot location in a field was chosen to represent an area where birds are most likely to forage based on the best adjacent avian habitat, highest potential for available seed by including turn-row area and adjacent field edge, and optimal blind location for observers to document behaviors without disrupting the birds.

For the first three consecutive days after planting, avian observations were carried out at each subplot by two observers who were experienced in identifying bird species in the region and familiar with normal avian behaviors and activities. These observations were carried out using binoculars and spotting scopes during morning hours that were considered to represent a period of high foraging activity (i.e., between 06:30 and 08:30 a.m.). One observer carried out avian census surveys, which consisted of identifying and recording the species and number of birds landing on the field planted with treated seed. The other observer monitored individual birds on the field until they departed and recorded the time spent on the field, the number of corn seeds consumed and the treatment status of any consumed corn (i.e., treated or waste corn from previous year). Since only birds of a certain minimum size are likely to consume corn seeds (Benkman and Pulliam, 1988; Diaz, 1990; Diaz, 1994), these behavioral observations focused on birds of sufficient size to consume corn seeds (e.g., red-winged blackbirds, grackles, blue jays, cardinals, crows, pheasants). Observers were instructed to record any mortality or impaired behavior if it occurred.

Video Monitoring Observations

In addition to the observations carried out using binoculars and spotting scopes during morning foraging hours described above, remote, time-lapse, video equipment was deployed to provide constant (24hr) surveillance of select areas flagged during the post-planting seed count due to the presence of treated seed spills or areas of multiple treated seeds remaining on the soil surface (i.e., where birds are most likely to encounter visible treated corn seeds). One to three of these areas were selected for video monitoring at 7 of the 11 London area fields and 5 of the 10 Guelph area fields, resulting in a total of 24 video monitored locations at 12 fields. At each of the video-monitored locations, well camouflaged cameras were set up and focused on the identified areas of treated seeds, which were counted prior to initiating the recording. The camera was then left to record until the next day (between 20 and 24 hours later), at which point the camera was stopped and the seeds were recounted. At most of the video-monitored locations, this process was repeated for three consecutive days, within the first three days after planting (see Supplemental Table S2). However, there were nine video-monitored locations that were monitored for shorter periods (one or two days) due to heavy rainfall that interfered with camera operability (see Supplemental Table S2). Likewise, at six locations, some monitoring occurred up to four days after planting due to weather conditions (see Supplemental Table S2). In total, 1,380 hours of video footage were collected. Following their retrieval from the fields, the videos were reviewed to determine if any birds or animals interacted with the visible treated seeds over the duration of the surveillance period.

Carcass Searches

All study sites were searched and cleared of existing animal carcasses just prior to, during, or immediately after planting. For the first three consecutive days after planting, post-planting carcass searches were conducted after completion of the daily on-site bird observations. These searches were conducted along the study plot boundaries and just outside the boundaries along the field borders. Carcass searches consisted of four investigators searching established transects by foot in the defined areas, which included the turn-row area of the fields. The outside boundaries were searched with four individuals lined up approximately 4 m apart with one person at the field edge and the remainder spaced out in the adjacent habitat. Moving counter-clockwise around the field, each person searched a 4-m band of ground to their right, covering approximately 16 m of edge habitat in one trip around the field. Investigators then lined up with one person at the field edge and the other three spaced 4 m apart toward the field center. Again, moving counter-clockwise, each person searched a 4-m band to their left around the field perimeter. This was repeated with the search line moving 16 m toward the field center for each additional pass around the field until the entire field was searched.

RESULTS

Analytical Verification of Clothianidin Concentration on Seeds

The nominal clothianidin application rate for the commercially available seeds under consideration in this study (i.e., Poncho® 1250, Bayer CropScience) was 1.25 mg active ingredient (a.i.) per seed. The mean measured loading per seed (± SD) from a representative sample of seeds used on the study fields was 1.3 ± 0.08 mg per seed (Table 2). The recovery in four QC samples was within 99.5% to 104% of the fortified concentration demonstrating proper performance of the analytical method.

Post-Planting Counts of Seeds on the Soil Surface

The estimated number of treated seeds that remained on each field ranged from 29 to 813 seeds/ha, with a mean (± SD) of 224 (± 167) (Table 3). This represents approximately 0.03 to 1.2% of the total sown seeds, with a mean (± SD) of 0.3% (± 0.2%), based on standard planting rates of 69,200 – 88,900 corn seeds per hectare (Ontario Ministry of Agriculture, Food and Rural Affairs, 2017). The variability between fields may relate to differences in planting equipment or soil characteristics (e.g., more seeds are likely to remain on the soil surface in heavy soil rather than light soil). The vast majority of seeds found on the soil surface after planting were found in the turn rows, and to a lesser extent along the first furrow down the edge of the field. The latter occurs because the outside planter wheel often rides on the untilled edge, which is rough and causes the planter to bounce more often than it does in the open field. Unincorporated corn was rarely found in the open field. Similarly, other field studies report a higher number of surface seeds on the headland of the field than at the center (Davis, 1974; Westlake et al., 1980; de Leeuw et al., 1995). Because each transect was set up to contain turn rows at one end and a field edge along one side, the ratio of turn-rows and edge rows to open-field (mid-field) was much higher than would be the case with a larger field. Therefore, the estimated mean number of treated seeds per hectare present on the soil surface on fields in this study is an over estimation of the mean number of seeds per acre on a larger field.

The estimated number of seeds missing on each field, determined on the recount the third day after planting, ranged from 0 to 136 seeds/ha, with a mean of 34.6 (± 36.8) (Table 3). This excludes London field L6, as this field was re-disked due to heavy rain washing out much of the planted corn, which buried the treated seeds that were originally counted on the soil surface immediately post planting. These missing seeds from all fields but L6, which represent approximately 0 to 0.2% of the total sown seeds, may include seeds that were removed by birds or foraging mammals. However, as indicated in Table 3, the sites with the greatest number of missing seeds experienced heavy rainfall. The number of missing seeds from sites experiencing heavy rain (i.e., 44-136 seeds/ha) was considerably higher than those without reported heavy rainfall (i.e., 0-26 seeds/ha) (Table 3). It is hypothesized that the greater number of seeds missing from the soil surface is due to the heavy rainfall burying seeds and not due to wildlife consumption. This hypothesis is supported by observations of changes to the field topography from the heavy rain reported by study personnel and the low number of treated seeds seen consumed by wildlife in the video recording and visual observations.

Characterization of Avian Species Visiting Study Fields and Observations of Avian Behavior on Treated Fields

During the overall avian census surveys, a cumulative total of 1,882 birds were observed to be present within the completely observable subplots of the London and Guelph region fields (one subplot per field). It was possible to characterize 1,863 of these birds into 36 unique species, while 13 could only be identified as sparrows (species not identifiable), and six birds could not be identified due to angle or lighting issues (see Supplemental Table S3). The European starling (*Sturnus vulgaris*) and common grackle (*Quiscalus quiscula*) composed 53% of the total birds observed on the study fields. Of the remaining species observed at the study plots, the next 13 most frequently sighted species accounted for an additional 43% of the sightings and the remaining 21 species accounted for 4% of the sightings. The complete census of bird species is presented in the Supplemental Table S3.

Detailed behavioral observations (e.g., time spent on the field and number of corn seeds consumed) of birds present within the completely observable subplots of the 21 fields (one subplot per field) were carried out for a total of 596 birds, representing 25 species, for a cumulative duration of 1,987 minutes (Table 4). The average duration spent on the treated fields by an individual observed bird was 3.3 minutes (Table 4). The European starling (*Sturnus vulgaris*), common grackle (*Quiscalus quiscula*), and red-winged blackbird (*Agelaius phoeniceus*) were the species most commonly observed on the treated fields and they spent an average of 2.5 minutes on the treated fields (Table 4). Additional details on individual bird observations are available in the Supplemental Table S4.

Of the 596 birds monitored during the behavioral observations, the majority of them (95%) did not consume any corn seeds while present on the observable subplots of each field. For those that did consume corn seeds, untreated waste corn left over from the previous season (much of which was still on the cob and had become soft due to over-wintering) was more frequently consumed than treated corn seeds (Table 5). Specifically, 21 birds, representing four different species, were observed picking through the waste seed until they found one that was soft enough to crush in their beaks, and then eating that seed or flying off with it (Table 5). In contrast, over the entire 1,987 minutes of individual bird observation, only two birds (one blue jay *[Cyanocitta cristata*] and one American crow [*Corvus brachyrhynchos*]) were observed to consume any clothianidin-treated corn seeds (Table 5). For both birds, this consumption was limited to a single treated seed (Table 5). Additionally, seeds with an unknown treatment status (i.e., for which treatment status could not be visually confirmed by the observer) were observed to be consumed by one Canada goose (*Branta canadensis*) (seven seeds), one blue jay (*Cyanocitta cristata*) (one seed) and one red-winged blackbird (*Agelaius phoeniceus*) (one seed) (Table 5).

The apparent preference of birds for untreated waste seed on the site may reflect their active avoidance of treated seed due to appearance, palatability, and/or a previous episode of post-ingestion distress (de Leeuw et al., 1995; Hartley et al., 2000; OECD, 1996; Avery et al., 1994; Lopez-Antia et al., 2014; Millot et al., 2017). Additionally, many bird species do not consume corn kernels whole, rather they work to crack the kernel and eat only the inner endosperm of the seed. The effort required to accomplish this with a hard, dried kernel is a deterrent from consuming corn seeds for many birds, which means that the presence of more attractive alternative food items is likely to decrease avian exposure to clothianidin-treated corn seeds in the field. Since waste from the previous growing season harvest is commonly available on the field when treated corn seeds are planted, the results of the current study provide a realistic indication of clothianidin-treated corn seed consumption in the field. In circumstances where waste seed is not available on the treated field, other food sources such as weed seeds on the field, seeds in hedge rows or off field areas, and invertebrates (in the case of omnivorous birds) are expected to be preferred to the hard, clothianidin-treated seeds.

No abnormal behaviors or mortalities were detected for any birds while present on the observable subplots of each field.

Video Monitoring Observations

Continuous video monitoring that focused on areas with the highest likelihood of avian exposure (i.e., treated seed spills or areas of multiple treated seeds remaining on the soil surface) was deployed at 24 locations across 12 fields (seven London area fields and five Guelph area fields). In total, 1,380 hours of video footage were collected and reviewed. Based on seed counts conducted before and after video monitoring, it was possible to observe that there were a total of three occasions when a single seed was removed from a monitored area, and one occasion when a seed was cracked, but not consumed (see Supplemental Table S2). A review of the collected video footage indicated that two of the three removed seeds were taken by mice. The third seed removal occurred during a heavy rain/wind storm, which caused the camera to malfunction. Therefore, it was not possible to identify whether the seed was displaced by the weather or by an animal. However, it was unlikely to have been removed by a bird because this removal occurred during the night, and granivorous birds do not generally feed at night. It was also not possible to determine whether an animal had handled the single observed cracked seed, because the camera was blown over by the wind during that observation period. Therefore, it is possible that a bird attempted to eat this seed but was ultimately deterred from consuming it. Overall, across 1,380 hours of video monitoring that focused on areas where multiple seeds were available on the soil surface, no birds were observed eating treated seed. Additionally, no abnormal wildlife behavior or mortalities were observed.

Carcass Searches

No bird carcasses were located during the carcass searches conducted on each field at the end of each day of daily bird observations (i.e., each day during the three-day post-planting observation period). The absence of bird carcasses during the three-day search period post-planting does not necessarily mean that mortality did not occur. It is generally recognized that several factors can contribute to inaccuracies in determining the number of bird mortalities based on recovered carcasses including: (1) birds may die outside of the treated field or the area; (2) carcasses may be removed from the search area by scavengers before they are detected (3) the efficiency of searchers at detecting carcasses and; (4) the duration over which monitoring is completed (Bird Studies Canada, 2017). However, these factors were unlikely to influence the results of these carcass searches. Symptoms of acute clothianidin toxicity (i.e., ataxia and lethargy which could prevent birds from fleeing the treated field after exposure) were not observed during any of the observations. The individuals who performed the carcass searches were trained experts and chronic toxicity is not expected based on the limited extent (magnitude and duration) of exposure.

DISCUSSION

The objectives of this study were 1.) to evaluate how many clothianidin-treated corn seeds remain available on the soil surface after planting and 2.) to observe how frequently (if at all) these remaining seeds were consumed by birds under realistic label use conditions.

With respect to the first objective, this study has demonstrated that a very small proportion (in most cases, less than 1.0%) of treated corn seed, remains available on the soil surface after planting following standard agronomic practices. These findings support the EPA (2016) recommendation that risk assessments for pesticide treated seeds could be refined by assuming that only 1% of seeds that are planted underground with an in furrow or drill seed planter (like corn) remain available on the surface for bird consumption. Based on the data available from the 21 fields monitored in this study, the 99% incorporation rate recommended by EPA (2016) is likely to provide a realistic upper limit (or overestimate) of the number of treated corn seeds that are available on the soil surface for bird consumption.

According to optimal foraging theory, birds will choose food items that will maximize their energy and nutrient uptake per unit time. If the food item is not abundant (density), the time spent searching for this item may be too high and other food items will be included in the diet. As such, areas with high seed density (seed spill sites) are more attractive for birds than areas with only irregularly scattered seeds (de Leeuw et al. 1995). For instance, Murton et al. (1963) found that food densities <2 grains/m^2 were too low for the wood pigeon to exploit successfully. Similarly, Moorcroft et al. (2002) found that grey partridges rarely feed on fields where cereal grain density was <50 seeds/m^2. Based on the mean number of available seeds per hectare (179-London and 273-Guelph), the mean seed density on the soil surface in the London and Guelph study area was 0.02 and 0.03 seeds/m^2, respectively. Thus, the densities of treated corn seeds available on London and Guelph study areas after sowing may not be sufficient to attract some birds.

With respect to the second objective, the results of this study indicate clothianidin treated corn seeds are rarely consumed by birds under normal agronomic practices. Both the on-site behavioral monitoring and video monitoring approaches were designed to maximize seed availability and attractiveness to birds (e.g., carried out in the first 3-4 days after planting, in areas adjacent to suitable avian habitat that encompass locations where treated seed is either more likely to be available or has already been observed). However, despite more than 1,900 minutes of on-site behavioral observation of 596 individual birds during morning hours that were considered to represent a period of high foraging activity (i.e., between 06:30 and 08:30 a.m.), only two birds were observed to consume any clothianidin-treated corn seeds and only three birds consumed seeds with an unknown treatment status (i.e., for which treatment status could not be visually confirmed by the observer) (Table 5). Similarly, no birds were observed to consume treated seeds during 1,380 hours of video monitoring of areas with the highest likelihood of avian exposure (i.e., treated seed spills or areas of multiple treated seeds remaining on the soil surface).

The primary route of exposure requiring risk evaluation for birds that may consume clothianidin-treated corn seeds is potential consumption of high numbers of recently planted seeds in a short timeframe. The potential for chronic exposure in the field is unlikely, considering the dissipation of the active ingredient from the seed and the rapid germination of commercial seeds. Additionally, chronic feeding on cached seeds is unlikely as the density of exposed seeds is too low for buildup of large caches. Therefore, for the small number of birds that did consume a few treated seeds or seeds with an unknown treatment status in this study, acute oral toxicity data for clothianidin can be used to illustrate the potential significance of the observed clothianidin exposure.

Quantitative acute oral toxicity data are available for four species of birds (see Supplemental Table S5), with the reported LD50s ranging from 414 to >2,000 mg a.i/ kg body weight. Based on the acute oral toxicity data for the most sensitive bird species tested (i.e., 414 mg a.i./kg bw, reported for the red-winged blackbird [*Agelaius phoeniceus*]) the number of treated seeds that would constitute an acute median lethal dose for the birds observed consuming treated seeds or seeds with an unknown treatment status was calculated and compared to the number of seeds that they were observed to consume (Table 6). Based on the observed treated seed consumption, one blue jay and one American crow were exposed to approximately 3.5% and 0.6%, respectively, of the LD50 dose (Table 6). Additionally, if it is assumed that all the consumed seeds of unknown status were actually treated seeds, then the exposure for the blue jay would increase to 7% of the LD50, and one red-winged blackbird and one Canada goose would also have been exposed to 5.2% and 0.5% of the LD50, respectively. This study does not provide a full account of the dietary exposure to clothianidin of individual birds for an entire day (i.e., the daily dose). However, given that most individual birds did not consume any treated seeds at all and that the treated seeds did not appear to be an attractive food item to birds (even in areas where seed density was highest/ most attractive), it is expected that overall clothianidin exposure due to consumption of treated corn seeds will be minimal for most birds.

CONCLUSION

The amount of pesticide ingested by birds foraging on treated seeds is largely dependent on the share of treated seeds in their diet (de Leeuw et al., 1995; Avery et al., 1997). Evaluations of avian risk from pesticide treated seeds are often based on the worst-case assumptions that 1.) all seeds will be available on the surface after planting, and 2.) birds will feed exclusively in the treated field, eating only treated seeds (i.e., 100% of their diet consists of treated seed). The results of this study demonstrate that these assumptions considerably overestimate exposure (and therefore risk) for clothianidin-treated corn planted according to standard agronomic practices. Based on this study, only a small proportion (in most cases, less than 1.0%) of treated corn seed remained available on the soil surface after planting, and this treated seed was not an attractive food source to birds.

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COMPLIANCE WITH ETHICAL STANDARDS

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Conflicts of Interest: Daiana Duca, Melissa Whitfield-Aslund, Nicole Kopysh, Tereza Dan, Loren Knopper are employees of Stantec Consulting Ltd., which provides consultation for Bayer CropScience. Smithers Viscient employed Larry Brewer at the time the study was conducted, and has received research funding from Bayer CropScience. Sean McGee is an employee of Bayer CropScience.

Animal Use: Guidelines for the care and use of animals were not applicable for this field observation study.

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Table 1(on next page)

Description of seed count transects at each studied field

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Field[a]	Seed Row Length (m)	Row Spacing (cm)	Total Number of Rows Included in Transect	Seed Count Transect Area (hectares)
L1	230	76.2	30	0.53
L2	300	48.3	50	0.73
L4	300	76.2	30	0.69
L5	300	76.2	30	0.69
L6	300	76.2	30	069
L7	400	48.3	30	0.57
L10	300	48.3	60	0.85
L11	300	76.2	30	0.69
L12	300	73.7	30	0.65
L13	276	76.2	30	0.65
L14	300	76.2	30	0.69
G1	250	73.7	30	0.57
G2	300	76.7	30	0.69
G3	300	76.7	30	0.69
G4	300	76.7	30	0.69
G5	250	76.7	30	0.57
G7	300	76.7	30	0.69
G10	250	76.7	30	0.57
G11	250	76.7	30	0.57
G12	250	76.7	30	0.57
G13	300	76.7	30	0.69

[a] L prefix = London area field, G prefix = Guelph area field

Table 2 (on next page)

Analytical verification of clothianidin loading on a representative subset of treated corn seeds

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Study Field	**Nominal Concentration** (mg per 50 seeds)	**Analytical Result** (mg per 50 seeds)	**Analytical Result** (mg per seed)	**Percent of Nominal Concentration**
L5	62.5	64.4	1.3	103
L2	62.5	67.1	1.3	107
L7	62.5	69.6	1.4	111
G2	62.5	67.9	1.4	108
G9	62.5	58.9	1.2	94.3
Mean		**65.6**	**1.3**	**105**
Standard Deviation		**4.17**	**0.08**	**6.7**

L prefix = London area sites, G prefix = Guelph area sites

Table 3 (on next page)

Post-planting soil surface seed count

Field[a]	Seed Count Transect Area (hectares)	Seeds on Transect Soil Surface Day 1 (#)	Estimated Seeds on Soil Surface per hectare Day 1	Seeds Missing from Transect Soil Surface Day 3 (#)	Estimated Seeds Missing per hectare Day 3
L1	0.53	19	35.8	3	5.66
L2*	0.73	146	200	32	43.8
L4	0.69	20	29.0	3	4.35
L5	0.69	159	230	7	10.1
L6*	0.69	154	223	No data[b]	N/A
L7	0.57	68	119	14	24.6
L10	0.85	241	284	17	20
L11*	0.69	133	193	37	53.6
L12*	0.65	185	285	40	61.5
L13	0.65	37	56.9	0	0
L14*	0.69	217	315	94	136
G1*	0.57	169	297	39	68.4
G2	0.69	57	82.6	5	7.25
G3*	0.69	208	301	61	88.4
G4*	0.69	561	813	33	47.8
G5	0.57	174	305	0	0
G7*	0.69	206	299	52	75.4
G10	0.57	69	121	3	5.26
G11	0.57	61	107	15	26.3
G12	0.57	69	121	0	0
G13	0.69	196	284	10	14.5
Mean	**0.65**	**150**	**224**	**23.3**	**34.7**
Standard Deviation	**0.08**	**117**	**167**	**25.1**	**36.8**

[a] L prefix = London area sites, G prefix = Guelph area sites

[b] Due to a severe thunderstorm which washed out much of the drilled corn seed from the furrows, the grower re-disked the field, and in doing so, buried all seed on the study site

(*) denotes sites that experienced heavy rainfall within the three-day post-planting period

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Table 4(on next page)

Most frequent bird species encountered during detailed behavioral observations

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1

Common Name	Number of individual birds observed			Cumulative time that birds were observed on a field (minutes)	Average time spent per bird (minutes)
	Guelph Fields	London Fields[a]	Total		
European starling	105	76	181	487	2.7
Common grackle	97	21	118	287	2.4
Red-winged blackbird	22	51	73	160	2.2
American robin	20	19	39	113	2.9
American crow	36	**0**	36	200.5	5.6
Pigeon (rock dove)	30	0	30	238	7.9
Brown-headed cowbird	0	16	16	33.5	2.1
Horned lark	15	0	15	37	2.5
Other[b]	28	60	88	431	4.9
Total	**353**	**243**	**596**	**1987**	**3.3**

[a] There were two London sites on which field observations were not possible on Day 2 and Day 3 (site L6) and on Day 3 (site L14) due to heavy rainfall and severe thunderstorms.

[b] 'Other' includes all species observed on a study plot fewer than 15 times during the observation period. For the London sites, this included a total of 21 species, each of which was observed fewer than 12 times. For the Guelph sites, this included a total of 8 species, each of which was observed fewer than 10 times

2

Table 5(on next page)

Summary of birds observed to consume seeds on the London and Guelph study sites during detailed behavioral observations

Test Site Location	Species	Number of Individual Birds Observed Consuming Seeds	Number of Treated Seeds Consumed	Number of Seeds Consumed with Unknown Treatment Status[b]	Number of Non-treated Seeds Consumed
London[a]	Red-winged blackbird	1	0	1	0
	Common grackle	1	0	0	5
	Brown-headed cowbird	1	0	0	1
Guelph	Blue jay	1	1	1	0
	Horned lark	1	0	0	2
	Canada goose	1	0	7	0
	Common grackle	9	0	0	>7[c]
	House sparrow	9	0	0	ND[d]
	American crow	1	1	0	0
	Total	**25**	**2**	**9**	**>15[e]**

[a] There were two London sites on which field observations were not possible on Day 2 and Day 3 (site L6) and on Day 3 (site L14) due to heavy rainfall and severe thunderstorms.

[b] Seed treatment status could not be visually confirmed

[c] Four of the nine common grackle consumed an undetermined amount of non-treated seeds

[d] ND - Not determined. The number of non-treated seeds consumed by the nine individual house sparrows could not be determined

[3] The total number of non-treated corn seeds consumed could not be determined as a result of the two bird species which consumed an indeterminate amount

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Table 6(on next page)

Clothianidin avian acute oral toxicity

1

Species	Body Weight (kg) [a]	Number of Treated Seeds Consumed	Number of Seeds Consumed with Unknown Treatment Status	Potential Exposed Dose [b] (mg a.i./kg bw)	Fraction of LD50 to which bird may have been exposed (%) [c]
Red-winged blackbird	0.06	0	1	21.7	0-5.2
Blue jay	0.09	1	1	28.9	3.5-7.0
American crow	0.55	1	0	2.36	0.6
Canada Goose	4.5	0	7	2.02	0-0.5

[a] For the red-winged blackbird, the body weight is represented by the median value of the body weight range reported by Rosenthal (2004); for the blue jay and American crow, values were taken from Poole, E. 1938; for the Canada goose value was taken from BC MOE, 1996

[b] = (seed dose*total number of seeds consumed)/Body weight[a.]. The seed dose is the mean measured clothianidin concentration per seed (1.3 mg a.i./seed)

[c] = Potential exposed dose[b]/the lowest available acute avian LD50 of 414 mg a.i./ kg-bw for the red-winged blackbird. If a range is shown, the lower limit represents only exposure via consumption of treated seeds and the upper limit represents estimated exposure if all seeds with unknown treatment status are assumed to be treated seeds.

2