Assessment of risk to hoary squash bees (Peponapis pruinosa) and other ground-nesting bees from systemic insecticides in agricultural soil

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Using the hoary squash bee (Peponapis pruinosa) as a model, we provide the first probabilistic risk assessment of exposure to systemic insecticides in soil for ground-nesting bees. To assess risk in acute and chronic exposure scenarios in Cucurbita and field crops, concentrations of clothianidin, thiamethoxam and imidacloprid (neonicotinoids) and chlorantraniliprole (anthranilic diamide) in cropped soil were plotted to produce an environmental exposure distribution for each insecticide. The probability of exceedance of several exposure endpoints (LC50s) was compared to an acceptable risk threshold (5%). In Cucurbita crops, under acute exposure, risk to hoary squash bees was below 5% for honey bee LC50s for all residues evaluated but exceeded 5% for clothianidin and imidacloprid using a solitary bee LC50. For Cucurbita crops in the chronic exposure scenario, exposure risks for clothianidin and imidacloprid exceeded 5% for all endpoints, and exposure risk for chlorantraniliprole was below 5% for all endpoints. In field crops, risk to ground-nesting bees was high from clothianidin in all exposure scenarios and high for thiamethoxam and imidacloprid under chronic exposure scenarios. Risk assessments for ground-nesting bees should include exposure impacts from pesticides in soil and could use the hoary squash bee as an ecotoxicology model.

Global insect pollinator declines are being driven by multiple interacting environmental stressors, including land-use intensification, pathogens, invasive species and climate change, and may threaten the production of crops that depend directly or indirectly on the pollination services that bees provide1,2. For bee populations living in proximity to agricultural production, exposure to pesticides is likely one of the major environmental stressors affecting population health2,3.

Cucurbita crops (e.g., pumpkin, squash, summer squash, and gourds) are grown globally for their fruits. Because of their imperfect flowers and heavy, oily pollen, they are dependent upon bees for pollination4. The insecticides used to control pests in Cucurbita crops can also harm beneficial insect pollinators, setting up a tension between the need to control pests while maintaining healthy bee populations for the essential pollination services they provide.

In Ontario, three neonicotinoids (imidacloprid, clothianidin, and thiamethoxam) are commonly used in Cucurbita-crop production to control insect pests5. Once applied, thiamethoxam breaks down quickly to clothianidin as one of its metabolites6,7. Although they are effective against pests, neonicotinoid insecticides are of environmental concern because of their relatively high toxicity to (non-target) insects, their systemic nature, their persistence, and their extensive use in agriculture8,9. In agriculture globally, about 60% of neonicotinoids are applied as seed coatings or as in-furrow soil applications, with the remaining applied as foliar sprays8. Neonicotinoid residues have been found in the nectar and pollen of Cucurbita flowers10,11, and in agricultural soil12, where they have been found to persist across seasons12,13.

For bees, both adult and larval stages may be exposed to pesticide residues consumed in nectar and pollen. The extent of exposure is likely greatest for individual adult female solitary bees because they consume pollen and nectar during sexual maturation and egg laying14–16, consume nectar to fuel their foraging and nesting activities, and handle pollen and nectar to feed their offspring15,17. Male solitary bees consume nectar and pollen during...
sexual maturation, and nectar thereafter to fuel flight. Solitary bee larvae consume and topically contact pollen and nectar in their provisions. For bees that do not construct their nests from glandular secretions, exposure may also be via nesting materials. For ground-nesting bees, exposure from nesting sites is via soil contacted during nest excavation for adult females, and via contact with the soil that forms nest cells during larval development. However, larval exposure within nest cells may be precluded because of the water-resistant coating applied to nest cells by many ground-nesting species. Adult male ground-nesting bees have little exposure to soil as they do not participate in nest construction, but they might have greater exposure to residues on plants than adult females because they may spend more time in flowers (e.g. residing inside them overnight).

The persistence of neonicotinoids in soil makes both short-term (acute) and long-term (chronic) contact exposure scenarios for ground-nesting bees plausible. Although neonicotinoid uptake from soil by bees has not yet been quantified, the translocation of neonicotinoids to bees from residues in dust generated during corn planting is well documented. Although their sublethal impacts on most wild bee species are unknown, exposure to neonicotinoids can affect the transmission of information through the nervous system of both adult and larval individuals. Such sublethal impacts on the normal physiology and behaviour of individual bees can have knock-on impacts for the function of social bee colonies and ultimately populations of both solitary and social species. Substantial knowledge gaps remain around the toxicity and effects of neonicotinoids to arthropods, including ground-nesting bees, in soil. Furthermore, exposure routes relating to nesting materials, including soil for ground-nesting bees, are not considered as part of current regulatory risk assessments for pesticide impacts on pollinators.

Hoary squash bees (Peponapis pruinosa) are solitary bees that build their nests in the ground (Fig. 1) within Cucurbita cropping areas and consume mostly Cucurbita-crop pollen and nectar. In eastern North America, hoary squash bees are one of the most important pollinators of Cucurbita crops and are obligately associated with these crops because they lack a wild plant host. In 2014, the Pesticide Management Regulatory Agency (PMRA) of Health Canada initiated a special review of registered neonicotinoid insecticides used on cucurbit crops because of concerns about their potential impacts on hoary squash bees. The hoary squash bee is common, nests in (sometimes large) aggregations, is a dietary specialist, has a well-documented natural history, and...
| INSECTICIDE | Contact | Oral | Mean Conc. in Matrix | HQ Adult Female | Mean Conc. in Matrix | HQ Larvae (Oral) | HQ Adult Female (Contact) | Mean Conc. in Matrix | HQ Adult Female | Across Exposure Matrix, All Stages | Adult Female | Larvae |
|-------------|---------|------|---------------------|----------------|---------------------|----------------|------------------------|---------------------|----------------|-------------------------------|--------------|------|
| Neonicotinoid | 3.35 g/bec | 0.06 | 0.03 | 0.45 | 4.92 | 4.86 | 0.06 |
| Pyraclostrobin | 1.69E-5 | 0.78 g/bec | 0.02 | 0.03 | 0.06 | 0.04 | 0.02 | 0.02 | 2.26E-4 |
| Chlorantraniliprole | 2.2E-5 | 0.03 | 0.03 | 0.06 | 0.04 | 0.04 | 3.99E-4 |
| Methomyl | 0.0542 g/bec | 0.06 | 0.03 | 0.06 | 0.04 | 0.06 | 0.04 | 0.02 | 2.26E-4 |
| Dimethoate | 0.01 | 0.5 | 2.71 | 0.04 | 2.26E-4 |
| Carbaryl | 0.08 | 0.78 g/bec | 0.02 | 0.03 | 0.06 | 0.04 | 0.04 | 0.007 | 0.0007 | 0.0006 |
| Thiamethoxam | 25.64 | — | ND | 0 | ND | 0 | 0 | 0 | 0 | 0 |
| Chlorantraniliprole | 3.99E-4 | 9.2E-5 | 0.03 | 0.03 | 0.06 | 0.04 | 0.04 | 0.007 | 0.0007 | 0.0006 |
| Pyraclostrobin | 3.8 | 2.2E-5 | 0.0542 g/bec | 0.03 | 0.03 | 0.06 | 0.04 | 0.04 | 0.02 | 2.26E-4 |
| Chlorantraniliprole | 0.03 | 0.03 | 0.06 | 0.04 | 0.04 | 3.99E-4 |
| Methomyl | 0.0542 g/bec | 0.06 | 0.03 | 0.06 | 0.04 | 0.06 | 0.04 | 0.02 | 2.26E-4 |
| Dimethoate | 0.01 | 0.5 | 2.71 | 0.04 | 2.26E-4 |
| Carbaryl | 0.08 | 0.78 g/bec | 0.02 | 0.03 | 0.06 | 0.04 | 0.04 | 0.007 | 0.0007 | 0.0006 |
| Thiamethoxam | 25.64 | — | ND | 0 | ND | 0 | 0 | 0 | 0 | 0 |
| Chlorantraniliprole | 3.99E-4 | 9.2E-5 | 0.03 | 0.03 | 0.06 | 0.04 | 0.04 | 0.007 | 0.0007 | 0.0006 |

**Table 1.** Hazard quotients (HQ) for each active ingredient, and pesticide type (insecticide, fungicide) in three exposure matrices (soil, pollen, nectar) and both developmental stages (adult female or larvae) based on honey bee LD50 values taken from the literature and mean residue concentration in the exposure matrices for all pesticide residues found detected on 18 Cucurbita-crop farms in Ontario in 2016. Numbers shown in bold face are combined hazard quotients for a pesticide type, an exposure matrix, or a developmental stage. Where “ND” is indicated, residues were not detected, where “NQ” is indicated, residues were not quantifiable in bold face are combined hazard quotients for a pesticide type, an exposure matrix, or a developmental stage.

is easy to maintain in captivity (D.S.W.C., personal observation), making it a promising candidate as a model species to evaluate risk of exposure to pesticides in soil for ground-nesting bees. Our aims are (1) to evaluate which pesticides and exposure matrices (soil, pollen, nectar) pose a potential hazard to hoary squash bees; (2) to determine which hoary squash bee developmental stage (adult female or larvae) is at greatest hazard; (3) to evaluate risk from agricultural soil for ground-nesting solitary bees; and (4) to contribute to risk assessments for hoary squash bees and other solitary bees.

As a first step in assessing risk, we evaluated the concentrations of all pesticides found in composite samples of soil (n = 29), nectar (n = 25), and pollen (n = 25) taken from 18 farms growing Cucurbita crops. For each pesticide, we related the geometric mean concentration of all samples with quantifiable concentrations to the honey bee lethal dose endpoint (LD50) of that pesticide to produce a hazard quotient (HQ). Hazard quotients were summed for each route of exposure, and bee developmental stage (Table 1). Hazard quotients ≥1 flag pesticides or scenarios of concern. For flagged pesticides, and a single relevant unflagged pesticide, we undertook a probabilistic risk assessment that included all data values (including those below the limit of quantification) to determine the probability of exceeding the lethal dose. We developed Environmental Exposure Distributions (EEDs) for chronic exposure (33.5 g soil over 30 days) and acute exposure (2.23 g soil over 48 h) scenarios using honey bee LD50 values taken from the literature and mean residue concentration in the exposure matrices for hoary squash bees and other solitary bees.

Results

**Pesticide residue profiles-Cucurbita crops.** Residues of 7 insecticides, 6 fungicides, and 2 herbicides were detected in samples taken from Cucurbita crops (Table S3). We did not assess herbicide residues further. The three exposure matrices (pollen, nectar, and soil) show different pesticide residue profiles (Table S3). In soil, 5/7 insecticides and 5/6 fungicides were detected. Pollen contained residues of 4/7 insecticides and 6/6 fungicides, but residues were detected much less frequently and at lower concentrations than in soil. Nectar contained 3/7 insecticides and 3/6 fungicides, with the lowest concentrations and frequency of detection. Imidacloprid was the only insecticide detected in all three matrices (21% of soil samples, 3% of pollen and nectar samples). Thiamethoxam
was present only in a single soil sample, at a concentration below the limit of quantification (LOQ). Clothianidin was detected in 34% of soil samples but was not detected in nectar or pollen. The insecticides chlorantraniliprole and carbaryl were detected in soil (24% and 10% of samples respectively) and pollen samples (3% and 7% respectively), but not in nectar. The insecticides methomyl and dimethoate were not detected in soil, but dimethoate was detected in pollen and nectar (3% each), and methomyl was detected in 7% of nectar samples. The fungicides pyraclostrobin and propamocarb were detected in all three matrices, whereas boscalid, quinoxyfen and difenoconazole were detected in soil and pollen only. The fungicide picoxytrob was only detected in nectar and pollen.

**Hazard assessment-hoary squash bee.** Pesticides. Hazard quotients (HQ) for fungicide and insecticide residues in soil, pollen, and nectar for adult females and larvae based on honey bee LD₅₀ values are presented in Table 1. As expected, the combined HQ for insecticides (HQinsecticide total = 4.92) was much higher in all exposure matrices than for fungicides (HQfungicide = 0.03). Only clothianidin and imidacloprid had HQs ≥ 1 in soil, and the combined HQ of all non-neonicotinoid insecticides in soil was low (HQnon-neonicotinoid = 0.06). Although chlorantraniliprole had an HQ < 1, it was included in further probabilistic risk assessment for comparison because, like neonicotinoids, it is systemic and was present in 24% of samples. When we calculated HQs based on a solitary bee surrogate LD₅₀ (HB LD₅₀/10; the same pesticides, routes of exposure and developmental stage were flagged as hazardous, with the exception of methomyl (HQ = 2.6 for adult bee exposure via nectar; Table S9). The physical, chemical and environmental fate properties of the flagged neonicotinoids and chlorantraniliprole are presented in Table S4.

**Exposure Matrices.** Hazard quotients for pollen (HQadult female and larvae = 0.09) and nectar (HQadult male = 0.45) were low relative to HQs for soil (Table 1). However, only adult female exposure was assessed for nectar as little information exists about the amount of nectar consumed by larvae. Low HQs for pollen and nectar reflect the low insecticide concentrations detected in these matrices. Imidacloprid was the largest contributor to hazard for pollen, and methomyl was the largest contributor for nectar (Table 1). As HQ < 1 for pollen and nectar, they were deemed non-hazardous for the lethal dose endpoint. This does not imply that there are no hazards associated with sublethal endpoints as these were not evaluated. The combined HQ for insecticides in soil was high (HQcombined soil = 4.38; Table 1). Soil HQ was mostly attributable to neonicotinoids (HQcombined soil neonicotinoids = 4.32; 4.32/4.38 = 99%).

**Developmental Stage.** Hazard is greater for adult females (HQadult female = 4.86) than for larval hoary squash bees (HQlarvae = 0.06), mostly because of the adult exposure to neonicotinoid residues in soil during nest construction (HQadult female soil = 4.32; Table 1).

**Probabilistic risk assessment.** Hoary Squash Bees in Cucurbita Crops. For the acute exposure scenario (2.23 g soil, 48 h) in soil, environmental exposure distributions (EEDs) for clothianidin showed exceedance below 5% for the geometric mean honey bee LC₅₀ and the lowest honey bee LC₅₀ endpoints. However, exceedance for the solitary bee surrogate LC₅₀ endpoint (28.3%) was relatively high (Fig. 2a). Imidacloprid showed exceedance below 5% for the geometric mean honey bee LC₅₀ (3.5%) and exceedances greater than 5% for both the lowest honey bee LC₅₀ (8.9%) and the solitary bee surrogate LC₅₀ (31.2%) (Fig. 2b). For the chronic exposure scenario (33.5 g soil, 30 days) in soil, exceedance for clothianidin in soil was above 5% for all exposure endpoints in <i>Cucurbita</i> crops (geometric mean honey bee LC₅₀ = 35.8%; lowest honey bee LC₅₀ = 44.3%; solitary bee surrogate LC₅₀ = 68.7%) (Fig. 3a). For imidacloprid, exceedance for all lethal endpoints was also high under the chronic exposure scenario (geometric mean honey bee LC₅₀ = 39.8%; lowest honey bee LC₅₀ = 57.8%; solitary bee surrogate LC₅₀ = 85.4%) (Fig. 3b). Exceedance under both the acute and chronic exposure scenarios for chlorantraniliprole in soil was below 5% for all lethal dose endpoints assessed (Fig. 3c). Because high limits of detection and quantification (LOD/LOQ) rendered no quantifiable residue samples, EEDs could not be fitted to data for thiamethoxam.

**Ground-Nesting Bees in Field Crops.** Clothianidin was detected in 96.34%, imidacloprid was detected in 10.97%, and thiamethoxam was detected in 81.48% of soil samples taken from Ontario field crops (n = 82)13. Hoary squash bee exposure amounts (2.23 g soil-acute exposure; 33.5 g soil-chronic exposure) were used as a surrogate for exposure to determine exceedance for ground-nesting bees generally. In the acute exposure scenario no endpoint for imidacloprid (Fig. S1) showed exceedance above 5%, and only the solitary bee surrogate LC₅₀ endpoint showed exceedance above 5% for thiamethoxam (25.6%; Fig. 4b). In contrast, for clothianidin the probability of exceedance was greater than 5% for all exposure endpoints (honey bee geometric mean LC₅₀ = 11.72%; honey bee lowest LC₅₀ = 27.25%; solitary bee surrogate LC₅₀ = 81.85%), suggesting that the risk to ground-nesting bees is high from clothianidin in field crops soils, even when exposure is acute (Fig. 4a). In the chronic scenario, probability of exceedance for clothianidin was very high for all exposure endpoints (honey bee geometric mean LC₅₀ = 87.68%; lowest honey bee LC₅₀ = 92.4%; solitary bee surrogate LC₅₀ = 98.82%; Fig. 5a). For thiamethoxam, probability of exceedance was also high (honey bee geometric mean LC₅₀ = 35.7%; honey bee lowest LC₅₀ = 37.36%; solitary bee surrogate LC₅₀ = 78.42%; Fig. 5b). Probability of exceedance for imidacloprid under the chronic exposure scenario was below 5% for the honey bee geometric mean LC₅₀ (4.16%) but exceeded 5% for the honey bee lowest LC₅₀ (5.89%) and the solitary bee surrogate LC₅₀ (9.24%) (Fig. S2; Table S6).

**Discussion**
Comparing the three exposure matrices, soil had the greatest number of neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) detected, whereas imidacloprid was the only neonicotinoid detected in pollen and nectar. The maximum concentration of imidacloprid detected in soil (41.6 ng/g) was substantially greater than in pollen (4.3 ng/g) or nectar (1.1 ng/g), because imidacloprid is applied directly to soil and can bind to soil particles65 (Table S3). Although thiamethoxam is more soluble than imidacloprid, it is quickly metabolized to...
clothianidin, which is much less soluble than either imidacloprid or thiamethoxam (Table S4). Dively and Kamel reported higher imidacloprid residue concentrations (60.9 ng/g for pollen, 7.4 ng/g for nectar) and greater frequencies of detection (92% of pollen samples; 88% of nectar samples) in the pollen and nectar of Cucurbita crops. However, the interval between application and sampling was shorter in their study (5 weeks) than in our study (8 weeks). Stoner and Eitzer also reported higher concentrations of imidacloprid and thiamethoxam in Cucurbita pollen and nectar than found here, likely because they included anther and nectary tissue in their samples.

The analysis of the hazard of pesticides to hoary squash bees in Cucurbita crops is based on LD50/LC50 for adult honey bees as this represents current regulatory standards for testing toxicity in bees. Neonicotinoids are often more toxic to bees when exposure is via ingestion, and solitary bees are more sensitive than either honey bees or bumble bees to oral exposure, therefore using oral honey bee LD50 values in this study may under-represent oral toxicity to hoary squash bees. Toxicity via contact exposure varies much less among species, thus honey bee contact LC50 values may adequately represent contact toxicity for adult female hoary squash bees. The evaluation of hazard to larval squash bees using adult honey bee LD50 or LC50 values in this study may underestimate hazard because of developmental stage differences in sensitivity to neonicotinoids. Contact lethal doses are higher than oral lethal doses for the residues we detected (Table 1).

Thiamethoxam appears to pose minimal hazard because residues were only detected in a single sample at concentrations below the limit of quantification. However, an absence of thiamethoxam residues may be the result of rapid metabolization to clothianidin, which was detected in our soil samples. Both clothianidin (HQ = 1.82) and imidacloprid (HQ = 2.76) pose a hazard in Cucurbita growing systems in Ontario because they are detected frequently, and their respective HQs exceed one when summed across all exposure matrices. Imidacloprid appears to be more hazardous in Cucurbita-crops than clothianidin based on this approach because it was found at higher concentrations in soil and was present in both nectar and pollen (Table S3). The combined hazard of
all neonicotinoids in the system was also high (HQcombinedneonicotinoid = 4.59), suggesting that hoary squash bee populations may be exposed to lethal concentrations of neonicotinoids in a worst-case scenario. Combined hazard from chlorantraniliprole in all matrices was low (HQ = 0.02), likely because it has a much higher LC50 than neonicotinoids.

There is common agreement that bees can be exposed to neonicotinoids from nectar and pollen18,20,21. Although neither pollen nor nectar were deemed hazardous here, HQs were higher for adult hoary squash bees via Cucurbita nectar (HQnectar = 0.45) than via pollen (HQpollen = 0.03). This was because adult exposure to pollen was considered to be contact rather than oral (as adult consumption of pollen was not evaluated), whereas adult exposure to nectar was oral and also likely an overestimation based on honey bee nectar consumption values. Although no hazard from pollen or nectar was found for the lethal dose endpoint, sublethal effects are still possible at these low HQs.

Currently, there are no studies that evaluate the risk to ground-nesting bees from direct exposure to neonicotinoids in soil, although some studies assess effects on other soil fauna17. Because both imidacloprid and thiamethoxam (which metabolizes to clothianidin) are applied to the soil in Cucurbita crops, and may persist in the soil for longer than a single growing season in Canada16, it is unsurprising that hazard to ground-nesting hoary squash bees from neonicotinoids in soil (HQsoil = 4.32) is much higher than even the combined hazard from

**Figure 3.** Environmental Exposure Distribution (EED) for chronic exposure to (a) clothianidin, (b) imidacloprid, and (c) chlorantraniliprole concentrations in soil samples taken from 0–15 cm depth in Cucurbita-crop fields in Ontario, 2016. Effects benchmark concentrations for chronic exposure (30 days, 33.5 g soil) for the hoary squash bee, Peponapis pruinosa (i.e. honey bee geometric mean LC50 = red vertical line, honey bee lowest LC50 = blue vertical dotted line, and the solitary bee surrogate LC50 = green vertical dashed line), are represented on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from one. Grey horizontal lines represent individual soil samples below the analytical limits of detection or quantification, and black dots indicate samples for which insecticide residues could be quantified. The black line indicates the line of best fit to the data via maximum likelihood estimation (MLE) with associated 95% confidence intervals (grey shading).
neonicotinoids in both pollen and nectar (HQ pollen + nectar = 0.27; Table 1). Therefore, soil appears to be the most important route of exposure to systemic pesticides for hoary squash bees.

The combined hazard from insecticides for adult female hoary squash bees from all exposure matrices (soil, pollen, and nectar) was high, with 93% of this hazard attributable to neonicotinoids in soil (Table 1). Hoary squash bees can construct more than one nest per season when environmental conditions (e.g. nectar and pollen resources, weather) permit17. However, female hoary squash bees in this study were already exposed to doses above lethal levels of both imidacloprid and clothianidin (HQs > 1) during the construction of a single nest, rendering it unlikely they could construct another. Indeed, under present soil conditions in Ontario Cucurbita crops, pesticide exposure may preclude the construction of even a single 5-cell nest in a season, even if all other conditions are favourable. Excluding exposure from nectar, which was not evaluated, hazard to larval stages was low (HQlarvae pollen = 0.06). It is reasonable to expect that hazard from nectar is also low for larval stages because their consumption of nectar is much less than for adult females as they do not undertake energy-expensive nest construction or foraging activities. For honey bees, adult pollen foragers consume 150% more nectar than larvae42.

Here we have assumed that larval hoary squash bees are protected from direct exposure to neonicotinoids in soil by the waterproof nature of the nest cell lining17, an assumption that requires further critical investigation.

The percentage translocation of insecticide residues from soil to bees is currently unknown. Our initial assumption was translocation at 100%, but recognizing the uncertainty around translocation rates, we also present exceedances for four alternative scenarios with lower rates (Table S8). Using a probabilistic approach for the hoary squash bee in the acute exposure scenario (48 h, 2.23 g soil; Fig. 2, Table S6) at 100% translocation, the probability of exceedance of the mean honey bee LC50 was below 5% for imidacloprid and clothianidin. However, for the solitary bee surrogate LC50 endpoint residues of both imidacloprid (31.2%) and clothianidin (28.3%) in soil exceeded 5% (Fig. 2, Table S6). In the chronic exposure scenario, the amount of exposure to soil (33.5 g soil, 30 days) was much greater, and exceedance for imidacloprid and clothianidin was greater than 5% for all lethal endpoints (Fig. 3a,b, Table S6). It is likely that at least some of the clothianidin found in Cucurbita crop soil is...
from the application of thiamethoxam to seeds6. Hilton et al.7 found that ~3–46% of the residues recovered from soil sampled more than 60 days after thiamethoxam application were the metabolite clothianidin. Further work on the fate of thiamethoxam in Cucurbita crop fields is needed to determine whether seed-applied thiamethoxam poses a risk to hoary squash bees via its metabolite, clothianidin. Exceedance for chlorantraniliprole was not greater than 5% for any exposure endpoint in either exposure scenario, suggesting that it did not pose a risk in Cucurbita-crop soils in 2016 (Fig. 3c, Table S6).

There were at least three issues generating uncertainty during our assessment of risk to hoary squash bees posed by neonicotinoid residues in soil. The lack of information about insecticide toxicity for this, or indeed any other solitary ground-nesting species, is a large knowledge gap. As hoary squash bees are similar in size to honey bees, they may be well represented by the available toxicity data for honey bees, especially for contact exposure which tends to vary less among species than oral exposure39. Secondly, although soil-applied neonicotinoids are known to elicit negative effects on Lepidoptera pupae43, Carabid beetles44, Hexapoda, Collembola and Thysanoptera that live in soil45, we could find no information on the extent to which insecticide residues in soil can pass through the cuticle or into spiracles of ground-nesting bees. We have assumed a worst-case scenario in which all the soil residues are translocated during exposure, but this is unlikely, although neonicotinoids have relatively low organic carbon-water partition coefficients (Table S4)46. However, even at 10% translocation, exceedances were greater than 5% for all imidacloprid endpoints, and also for the lowest honey bee LC50 and solitary bee surrogate LC50 in both acute and chronic scenarios (Figs 2 and 3). Despite its limitations, this study is significant because it is the first evaluation of risk from insecticide residues in soil for any ground-nesting bee species.

**Figure 5.** Environmental Exposure Distribution (EED) for chronic exposure (30 days, 33.5 g soil) to (a) clothianidin and (b) thiamethoxam in soil from field crops (corn, soybeans, wheat) based on a government dataset35. Soil samples were taken from 0–15 cm depth in southern Ontario, 2016. Effects benchmark concentrations are for solitary ground-nesting bees based on the hoary squash bee (Peponapis pruinosa) exposure model. Effect benchmarks (i.e. honey bee geometric mean LC50 = red vertical line, honey bee lowest LC50 = blue vertical dotted line, and the solitary bee surrogate LC50 = green vertical dashed line) are represented on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from one. Grey horizontal lines represent individual soil samples below the analytical limit of detection, and black dots indicate samples for which insecticide residues could be quantified. The black line indicates the line of best fit to the data via maximum likelihood estimation (MLE) with associated 95% confidence intervals (grey shading).
Because little information about exposure to soil for most ground-nesting bee species exists, we used hoary squash bee exposure to soil as a surrogate for other ground-nesting bees. We estimated soil exposure of 33.5 g for hoary squash bee females, which is comparable to data for some other ground-nesting bee species. For example, soil exposure for *Andrena prunorum* or *Nomia melanderi* females are estimated as 30.23 g and 26.3 ± 5.7 g respectively. The difference between the low-end estimate of soil excavated by *N. melanderi* (20.6 g) and hoary squash bees (33.5 g) could represent as much as 38.5% of the latter species’ exposure, highlighting the potential variability in exposure via soil across species and the limitations of the hoary squash bee model. Ground-nesting bee species vary greatly in size, and many are much smaller than hoary squash bees. For ground-nesting solitary bees, tunnel diameter is related to bee size because bees excavate tunnels and nest cells large enough for themselves. Although other solitary ground-nesting bee species may be appreciably larger or smaller than squash bees, the ratio of their body size to the volume of soil they excavate when building a nest may be similar, providing a possible future basis upon which to compare exposure for different solitary bee species. Smaller bees may have lower exposure to neonicotinoids in soil because they construct narrower tunnels and nest cells to fit their smaller bodies, therefore contacting lower soil volumes overall. However smaller bee species may be more sensitive to insecticide exposure. Bee body size is not necessarily correlated to the depth that vertical tunnels in nests are excavated in soil. Solitary bees are physiologically limited in their reproductive capacity and generally build 1–8 brood cells per nest. Until more information emerges, the hoary squash bee is the best model available to evaluate risk from exposure to pesticide residues in soil for ground-nesting bees in general and provides a starting point to understand risk from insecticides residues in soil for these bees.

Neonicotinoid residues detected in Ontario agricultural soils reflect variation in usage for different crops. For *Cucurbita* crops, imidacloprid and clothianidin were the most commonly detected neonicotinoids, with a single detection of thiamethoxam. For field crops, clothianidin and thiamethoxam were more commonly detected. Exposure to clothianidin residues for bees nesting in field crop soils appeared to be ubiquitous: 96.34% of soil samples taken before spring planting contained clothianidin applied in the previous season. The probabilities of exceedance were high for all clothianidin exposure endpoints for both the acute (Fig. 4) and chronic (Fig. 5) scenarios. As clothianidin-treated seeds are planted in a new cropping cycle, releasing more residues into the soil, these exceedances would likely increase. About 70% of the solitary bee species in eastern Canada nest in the ground, many of which are associated with agriculture, including species in the genera *Agapostemon*, *Andrena*, *Anthophora*, *Colletes*, *Eucera*, *Halictus*, *Lasiosglossum*, *Megachile* and *Melissodes*. Ground-nesting bees from 13 genera have been collected in corn and soybean fields in Iowa. Although this does not prove that these species were nesting within fields, the small foraging ranges of solitary bees suggest that many likely were. Various bees are active at different times during the season. Those species active in the early spring may be exposed to the minimum residue concentrations described here, but those active post-planting may be exposed to much higher concentrations in soil.

Together, this evidence suggests the risk to ground-nesting bees from exposure to clothianidin in field crop soil is high, necessitating action to mitigate such risks to preserve pollination services. If clothianidin residues found in soil are metabolites of applied thiamethoxam, then use of thiamethoxam should also be addressed.

Thiamethoxam is used as a seed treatment on both field corn and soybean crops in Ontario and was applied to 1.98 million acres (66% of treated acres) in the 2016 season. For the chronic exposure scenario, the risk to ground-nesting bees from thiamethoxam was high for all exposure endpoints (Fig. 5b). For the acute exposure scenario, the risk from thiamethoxam was less than 5% for all exposure endpoints except the solitary bee surrogate LC50 (25.56%; Fig. 4b). The apparently lower risk to ground-nesting bees from thiamethoxam may be the product of its tendency to break down quickly into clothianidin in soil. Although the risk to ground-nesting bees from acute exposure to imidacloprid in field crop soil was below the 5% threshold for all exposure endpoints (Fig. S1), exceedance rose above the threshold for the solitary bee surrogate LC50 (9.24%) under the chronic exposure scenario (Fig. S2). The lower risk associated with imidacloprid in field crop soil may be because it is used in only 11% of treated field crop acres. One of the main concerns around neonicotinoid insecticide exposure for ground-nesting bees is their use in soil applications as treated seed. This has been partially mitigated in Ontario by increased regulation of neonicotinoid-treated corn and soybean seed but has not yet been addressed for other crops. In conclusion, neonicotinoid residues in soil pose a high risk to female hoary squash bees as they construct their nests in *Cucurbita*-crop growing systems or in field crop soils. These demonstrable risks for hoary squash bees seem likely to be applicable to other species of ground-nesting bees nesting in agricultural soils. Further work is needed to determine the relative sensitivity of the hoary squash bee to neonicotinoid exposure compared to honey bees, and to explicitly determine the extent and impacts of larval exposure in soil. Advances in analytical techniques are also needed to achieve lower limits of detection in soil that mirror lethal endpoints for solitary bees. Recognition and mitigation of risks from exposure to neonicotinoids in agricultural soil are urgently needed to protect these important crop pollinators.

**Methods**

In 2016, samples of soil (n = 29), nectar and pollen (n = 25) were taken from 18 *Cucurbita*-crop farms in Ontario. Ten soil samples (15 cm deep) per farm were combined and sub-sampled to produce a single 3 g sample for residue analysis. Pollen and nectar samples were collected directly from flowers. Nectar was harvested into a single 2 mL tube using a 20 µL micro-pipette. Pollen was collected from staminate flowers, weighed and put into 2 mL tubes. To determine the number of pollen grains per staminate flower, forty full anthers were placed individually into 2 mL microcentrifuge tubes each containing 0.5 mL of 70% alcohol. Pollen was dislodged from anthers by centrifuging at 2500 rpm for 3 minutes, and the tubes were topped up to 2 mL with 50% glycerin solution. The suspension was mixed with a mini vortex mixer and the number of pollen grains in five 5 µL aliquots were counted on a grid under 25X magnification. The mean number of pollen grains per 5 µL aliquot was then related back to the full 2 mL volume. The mass of a single *Cucurbita* pollen grain was determined by dividing the mean mass of...
pollen grains per anther (mean $\pm$ sd = 0.0302 $\pm$ 0.0211 g; n = 25) by the mean number of pollen grains per anther (mean $\pm$ sd = 1.8438 $\pm$ 0.9810; n = 40) (0.0302 g/18438 pollen grains $= 1.64 \times 10^{-6}$ g/pollen grain).

Information from the literature and this study were used to determine the realistic amount of pollen, nectar, and soil that adult female hoary squash bees would be exposed to via contact or ingestion, or that larvae would be exposed to via ingestion (Table S2). Males were not included in the evaluation, but are likely less exposed than females because they do not construct or provision nests\textsuperscript{37}. The amount of pollen consumed by larvae (0.0542 g pollen/nest cell) was calculated by multiplying the mass of a single pollen grain (as calculated above $= 1.64 \times 10^{-6}$ g) by the mean number of pollen grains in hoary squash bee larval provisions (33045.4 $\pm$ 12675.2 pollen grains)\textsuperscript{30}. Larval exposure to pesticide residues via contact with pollen in provisions was not evaluated. Contact exposure for adult females was assessed to be five times that for each larva because on average females provision five cells per nest\textsuperscript{17}. The amount of pollen and nectar ingested by adult females is unknown. However, for pollen-collecting honey bee workers, which are most like adult female hoary squash bees in their foraging behaviour, nectar consumption has been estimated at 10.4 mg sugar/day to fly to and within foraging patches\textsuperscript{42}. Based on this assumption, and the knowledge that Cucurbita pepo nectar is typically 40% sugar by volume\textsuperscript{30}, we assessed that each female hoary squash bee would consume 312 mg sugar in 780 mg of Cucurbita nectar to meet their energy requirements over 30 days. This is likely an overestimation as the foraging radius of squash bees from nest to flower patch is much smaller than for honey bees (honey bee foraging radius: mean $= 5.5$, maximum $= 15$ km\textsuperscript{60}; oligolectic solitary bee foraging radius $< 260$ m\textsuperscript{69}). Using hoary squash bee nest dimensions\textsuperscript{17}, the volume of soil excavated by a female bee during nest construction is 25.19 cm\textsuperscript{3} (Table S1). Multiplying the volume by the bulk density (BD) of loam soil, a common agricultural soil (BD\textsubscript{loam} $= 1.33$ g/cm\textsuperscript{3})\textsuperscript{61}, gives the mass of soil that a female hoary squash bee contacts as she constructs a nest with 5 cells over 30 days (25.19 cm\textsuperscript{3} $\times 1.33$ g/cm\textsuperscript{3} $= 33.51$ g). Acute (48 h) exposure was calculated by dividing chronic exposure by 15 (33.5 g/15 $= 2.23$ g; Table S2).

All samples collected from Cucurbita farms were submitted for analysis to University of Guelph Agri-Food Laboratories (Table S3) (ISO/IEC 17025 accredited). Pesticides were extracted using the QuEChERS Method\textsuperscript{82}. Samples were analyzed using their TOPS-142 LC pesticide screen, modified from the Canadian Food Inspection Agency (CFIA) PMR-006-V1.0 method including high performance liquid chromatography paired with electrospary ionization and tandem mass spectrometry and gas chromatography paired with tandem mass spectrometry.

Honey bee lethal effect endpoints (e.g., the concentration causing 50% mortality, LD\textsubscript{50} or LC\textsubscript{50}) were used for hoary squash bees because current regulatory standards consider the honey bee to be an adequate proxy for all bee species\textsuperscript{16,33}. Lethal doses for larval stages are rarely available\textsuperscript{20,22}. Honey bee LD\textsubscript{50} and LC\textsubscript{50} values were obtained from the US-EPA Pesticide Ecotoxicity Database\textsuperscript{63} and published literature. A geometric mean of multiple LC\textsubscript{50} values, reported from many sources\textsuperscript{32,38,63–68} and the lowest reported LC\textsubscript{50} value were used in this study. For contact with soil and pollen during nest construction and provisioning, contact honey bee LC\textsubscript{50} values were used. Oral honey bee LD\textsubscript{50} values were used for adult female hoary squash bee ingestion of nectar, and larval ingestion of pollen. For probabilistic risk assessment of soil exposure, the same lethal effect endpoints and a surrogate solitary bee contact LC\textsubscript{50} (honeybee LC\textsubscript{50}/10)\textsuperscript{33,34} were used. Lethal effect endpoints were converted to soil exposure concentrations as follows:

$$\text{Exposure Concentration Benchmark, (ng a.i. / g matrix)} = \frac{\text{Lethal Dose Effect Endpoint (ng a. i. /bee)}}{\text{Amount of soil exposure (g matrix/bee)}}$$

Hazard quotients (HQ) were calculated for each residue detected in each exposure matrix as follows:

$$\text{HQ} = \frac{\text{Concentration of residue in matrix}(\text{Amount of exposure to matrix})}{\text{Honey bee LD50 or LC50}}$$

An HQ $> 1$ indicates a potential lethal hazard. For HQs, the residue concentrations were calculated by taking the geometric mean of quantifiable concentrations in samples only. Concentrations of residue were reported in ng of active ingredient (a.i.) per g matrix, amount of exposure to a matrix was reported in g matrix/bee, and LD\textsubscript{50} or LC\textsubscript{50} were reported in ng a.i./bee. Hazard quotients were summed for each pesticide type (fungicide or insecticide), each exposure matrix (soil, pollen, and nectar), and each developmental stage (adult female or larva). Hazard quotients were also calculated based on a solitary bee surrogate LD\textsubscript{50} (HB LD\textsubscript{50}/10) (Table S9). After determining HQs, the hoary squash bee exposure models for both acute (48 h, 2.23 g soil) and chronic (30 days, 33.5 g soil) scenarios were used to carry out probabilistic risk assessment for both the hoary squash bee in Cucurbita crop soils using data from our own samples and for ground-nesting solitary bee species generally in field crops soils using a publicly-available dataset provided by the Ontario government\textsuperscript{13}. The government data set reported neonicotinoid residues in soil (15 cm depth) from 38 agricultural sites in Ontario in 2016, with a limit of detection of 0.05 ng/g for clothianidin, imidacloprid, and thiamethoxam. Insecticide residues in our samples represented field-realistic exposure for hoary squash bees on Cucurbita farms in 2016. Neonicotinoid residues in the government samples represented residues persisting in soil from a previous cropping cycle, rather than active ingredients applied in 2016. Environmental exposure distributions (EEDs) were constructed for each neonicotinoid in each exposure scenario and each crop type. Chronic exposure scenarios are reasonable for neonicotinoids because their effect on the nicotinic acetylcholine receptors (nAChRs) of insects are cumulative and irreversible\textsuperscript{64}. Although an EED for chronic exposure to chlorantraniliprole was constructed, evidence from honey bees suggests its effects may be transient as honey bees dosed orally or topically under artificial test conditions became lethargic but recovered within 48–72 hours\textsuperscript{68}.

EEDs were generated from the sample concentrations by fitting a log-normal or gamma distribution to the data via maximum likelihood estimation (MLE), using the fitdistirplus\textsuperscript{89,70}.​
This function allows for the fit of censored (right-, left-, or interval-censored) data enabling the use of the available “non-detect” (samples containing residues below the limit of detection (LOD)), and interval (samples containing residues above the limit of detection (LOD) but below the limit of quantification (LOQ)) data. The fitdistrplus package in R uses the probability plotting position function constructed for a given data set. The probability of exceeding the threshold (or the probability of exposure to an exposure concentration benchmark) is calculated as 1.00 minus the log-normal distribution for imidacloprid. EEDs were not constructed for thiamethoxam as there were no quantifiable residue data. For field crops soils, the best fit for all residue data was the gamma distribution. Percent exceedance (or the probability of exposure to a concentration benchmark) is calculated as 1.00 minus the cumulative probability. Exceedance lower than 5% was considered acceptable risk because it assures 95% protection of the population. Benchmarks were exposure concentrations associated with the lethal dose endpoints (geometric honey bee LC50, lowest honey bee LC50, solitary bee surrogate LC50). Table S5 summarizes a scenario assuming 100% translocation of residues from soil to bees (Table S6). As residue translocation from soil to bee is likely lower than 100%, exceedances assuming 10, 25, 50, 75% translocation are also presented (Table S8).

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Calderone, N. W. Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992–2009. PLoS One 7, e37235, https://doi.org/10.1371/journal.pone.0037235 (2012).
2. Vanbergen, A. J. et al. Threats to an ecosystem service: pressures on pollinators. Frontiers in Ecology and the Environment 11, 251–259, https://doi.org/10.1890/120126 (2013).
3. Krupke, C. H., Hunt, G. J., Etter, B. D., Andino, G. & Given, K. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One 7, e29268, https://doi.org/10.1371/journal.pone.0029268 (2012).
4. Whitaker, T. W. & Davis, G. N. Cucurbit: botany, cultivation, and utilization. 250 pp. (Interscience Publishers, 1962).
5. OMAFRA. Ontario Crop IPM: Cucumber Beetles Accessed March 20, 2018 from, www.omafra.gov.on.ca/IPM/english/cucurbits/insects/cucumber-beetles.html (2009).
6. Bonnatt, J. M. et al. Environmental fate and exposure: neonicotinoids and fipronil. Environmental Science and Pollution Research 22, 15–67, https://doi.org/10.1007/s11356-014-3332-7 (2015).
7. Hilton, M. J., Jarvis, T. D. & Ricketts, D. C. The degradation rate of thiamethoxam in European field studies. Pest Management Science 72, 388–397, https://doi.org/10.1002/ps.4024 (2016).
8. Jeschke, P., Nauen, R., Schindler, M. & Elbert, A. Overview of the status and global strategy for neonicotinoids. Journal of Agricultural and Food Chemistry 59, 2897–2908, https://doi.org/10.1021/jf101503g (2011).
9. Godfray, H. C. An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology 50, 977–987, https://doi.org/10.1111/1365-2664.12111 (2013).
10. Dively, G. P. & Kamel, A. Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. Journal of Agriculture and Food Chemistry 60, 4449–4456, https://doi.org/10.1021/jf205393x (2012).
11. Stoner, K. A. & Etter, B. D. Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (Cucurbita pepo). PLoS One 7, e39914, https://doi.org/10.1371/journal.pone.0039914 (2012).
12. Jennes, A., Harrington, P. & Turnbull, G. Neonicotinoid concentrations in arable soils after seed treatment applications in preceding crop years. Pest Management Science 70, 1780–1784, https://doi.org/10.1002/ps.3836 (2014).
13. MOECC. Soil neonicotinoid monitoring study Accessed Feb 3, 2018 from, https://www.ontario.ca/data/soil-neonicotinoid-monitoring-study (2016).
14. Michener, C. D. The Bees of the World, 2nd ed. 300 pp. (Interscience Publishers, 1962).
15. Cane, J. H. Adult pollen diet essential for egg maturation by a solitary Osmia bee. Journal of Insect Physiology 95, 105–109, https://doi.org/10.1016/j.jinsphys.2016.09.011 (2016).
16. Gogala, F. et al. Pesticide exposure assessment paradigm for solitary bees. Environmental Entomology 48, 22–35, https://doi.org/10.1093/ee/nvy105 (2019).
17. Mathewson, J. A. Nest construction and life history of the eastern cucurbit bee, Pepsis pruinosa (Hymenoptera: Apoidea). Journal of the Kansas Entomological Society 41, 255–261 (1968).
18. EFSA. Panel on Plant Protection Products and their Residues. Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (Apis mellifica, Bombus spp. and solitary bees). EFSA Journal 10, 2668, https://doi.org/10.2903/j.efsa.2012.2668 (2012).
19. Girolami, V. et al. Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers. Journal of Applied Entomology 133, 35–44, https://doi.org/10.1111/j.1439-0418.2012.01718.x (2013).
20. Godfray, H. C. J. et al. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proceedings of the Royal Society B-Biological Sciences 281, 20140558, https://doi.org/10.1098/rspb.2014.0558 (2014).
21. Godfray, H. C. J. et al. A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proceedings of the Royal Society B-Biological Sciences 282, 20151821, https://doi.org/10.1098/rspb.2015.1821 (2015).
22. Alkassab, A. T. & Kirchner, W. H. Subletal exposure to neonicotinoids and related side effects in insect pollinators: honeybees, bumblebees, and solitary bees. Journal of Plant Diseases and Protection 124, 1–30, https://doi.org/10.1007/s11364-016-0041-0 (2017).
23. Baron, G. L., Jansen, V. A. A., Brown, M. J. F. & Raine, N. E. Pesticide reduces bumblebee colony initiation and increases probability of population extinction. Nature Ecology & Evolution 1, 1308–1316, https://doi.org/10.1038/s41559-017-0260-1 (2017).
24. Julier, H. E. & Roulin, T. H. Wild bee abundance and pollination service in cultivated pumpkins: farm management, nesting behavior and landscape effects. Journal of Economic Entomology 102, 563–573, https://doi.org/10.1603/029.102.0214 (2009).
25. Hurd, P. D., Jr., Linsley, E. G. & Michelbacher, A. E. Ecology of the squash and gourd bee, Pepsis pruinosa, on cultivated cucurbits in California (Hymenoptera: Apoidea). Smithsonian Contributions to Zoology 168, 1–17 (Smithsonian Institution Press, 1974).
26. Willis, D. S. & Kevan, P. G. Foraging dynamics of Peponapis pruinosa (Hymenoptera: Anthophoridae) on pumpkin (Cucurbita pepo) in southern Ontario. Canadian Entomologist 127, 167–175, https://doi.org/10.4039/Ent127167-2 (1995).

27. Arntz, D. R. & Nault, B. A. Performance of Apis mellifera, Bombus impatiens, and Peponapis pruinosa (Hymenoptera: Apidae) as pollinators of pumpkin. Journal of Economic Entomology 104, 1153–1161, https://doi.org/10.1603/EC10431 (2011).

28. López-Uribre, M. M., Cane, J. H., Minckley, R. L. & Danforth, B. N. Crop domestication facilitated rapid geographical expansion of a specialist pollinator, the squash bee Peponapis pruinosa. Proceedings of the Royal Society B-Biological Sciences 283, 20160443, https://doi.org/10.1098/rspb.2016.0443 (2016).

29. PMRA. Re-evaluation Note REV2014-06. Special Reviews: potential environmental risk related to Peponapis pruinosa exposure to Clothianidin, Imidacloprid and Thiamethoxam used on curbzaches. Available from, https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/decisions-updates/re-evaluation-note/2014/peponapis-pruinosa-exposure-clothianidin-imidacloprid-thiamethoxam-rev2014-06.html (2014).

30. Williss, D. S. The pollination system of Cucurbita pepo and Peponapis pruinosa in southern Ontario. MSc. dissertation, University of Guelph, Ontario, Canada (1991).

31. Selim, H. M., Jeong, C. Y. & Elbana, T. A. Transport of Imidacloprid in soils: miscible displacement experiments. Soil Science 175, 375–381, https://doi.org/10.1097/SS.0b013e3181ece92 (2010).

32. EIPAC. Conclusion the peer review of the pesticide risk assessment for bees for the active substance thiamethoxam. EFSRA Journal 11, 3667, https://doi.org/10.2939/efs3.2013.3067 (2013).

33. US-EPA. Guidance for assessing pesticide risks to bees. Office of Chemical Safety and Pollution Prevention Office of Pesticide Programs Environmental Fate and Effects Division, Environmental Protection Agency, Washington DC; Environmental Assessment Directorate, Pest Management Regulatory Agency, Health Canada, Ottawa, CN; California Department of Pesticide Regulation. Available from https://www.epa.gov/sites/production/files/2014-06/documents/pesticide_risk_assessment_guidance_06_19_14.pdf (2014).

34. Arena, M. & Sgolastra, F. Meta-analysis comparing the sensitivity of bees to pesticides. Ecotoxicology 23, 324–334, https://doi.org/10.1016/j.ecotox.2014.01.003 (2014).

35. Rundlöf, M. et al. Pollinator risk assessment and pesticide risk management. A review. Functional Ecology 25, 555–564, https://doi.org/10.1111/j.1365-2435.2010.01641.x (2011).

36. Devillers, J. Comparative toxicity and hazards of pesticides to Apis mellifera. Environmental Toxicology and Chemistry 20, 2482–2486, https://doi.org/10.1897/2000.20.5.2482 (2001).

37. Suchail, S., Guez, D. & Belzunces, L. P. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in A. mellifera. Apidologie 37, 371–378, https://doi.org/10.1051/apido:2004071 (2005).

38. Marder, A. & Devillers, J. Ecotoxicity of neonicotinoid insecticides to bees. In: Insect Nicotinic Acetylcholine Receptors. Thany SE (ed.). Landes Bioscience/Springer Science & Business Media, LLC dual imprint/Springer series: Advances in Experimental Medicine and Biology 683, 96–106 (2012).

39. Atkins, E. L. D. & Kellum, D. Comparative morphogenic and toxicity studies on the effect of pesticides on honeybee brood. Journal of Apicultural Research 25, 242–255, https://doi.org/10.1080/002188398.1986.11100725 (1986).

40. Miliczky, E. Observations on the nesting biology of Andrena (Plastandrena) pruinosa, Cockerell in Washington State (Hymenoptera: Andrenidae). Journal of the Kansas Entomological Society 81, 110–121, https://doi.org/10.2317/JKES-61121.1 (2008).

41. Cane, J. H. Annual displacement of soil in nest tumuli of alkali bees (Andrena: Andrenidae) across an agricultural landscape. Journal of the Kansas Entomological Society 76, 172–176 (2003).

42. Devillers, J. et al. Comparative toxicity and hazards of pesticides to Apis and non-Apis bees. A chemometrical study. SAR and QSAR in Environmental Research 14, 389–403, https://doi.org/10.1080/106293603101623980 (2003).

43. Peck, D. C. & Olmstead, D. Neonicotinoid insecticides disrupt predation on the eggs of turf-infesting scarab beetles. Bulletin Entomological Research 100, 689–700, https://doi.org/10.1080/00074831000000040 (2010).

44. Wettstein, F. E. et al. Leaching of the neonicotinoids thiamethoxam and imidacloprid from sugar beet seed dressings to subsurface tile drains. Journal of Agricultural and Food Chemistry 64, 6407–6415, https://doi.org/10.1021/jf503685z (2016).

45. Miliczky, E. Observations on the nesting biology of Andrena (Plastandrena) pruinosa, Cockerell in Washington State (Hymenoptera: Andrenidae). Journal of the Kansas Entomological Society 81, 110–121, https://doi.org/10.2317/JKES-61121.1 (2008).

46. Cane, J. H. Annual displacement of soil in nest tumuli of alkali bees (Nomia melanderi) (Hymenoptera: Apiformes: Halictidae) across an agricultural landscape. Journal of the Kansas Entomological Society 76, 172–176 (2003).

47. Devillers, J. et al. Comparative toxicity and hazards of pesticides to Apis and non-Apis bees. A chemometrical study. SAR and QSAR in Environmental Research 14, 389–403, https://doi.org/10.1080/106293603101623980 (2003).

48. Cane, J. H. Soils of ground-nesting bees (Hymenoptera: Apoidea): texture, moisture, cell depth and climate. Journal of the Kansas Entomological Society 64, 406–413 (1999).

49. Packer, L., Genaro J. A. & Shepherd C. S. The Bee Genera of Eastern Canada. Canadian Journal of Arthropod Identification No. 3, 25 September 2007, https://doi.org/10.3752/cja.2007.03 (2007).

50. Toral-Grande, A., Mullen, E. K., Tonge, M. B., Guzman-Novoa, E. & Raine, N. E. Status and Trends of Pollinator Health in Ontario. University of Guelph report prepared for Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 238 pages. Accessed from https://rainlab.files.wordpress.com/2015/12/status-and-trends-of-pollinator-health-in-ontario-march-8-2017-tagged.pdf (2017).

51. USDA. Attractiveness of agricultural crops to pollinating bees for the collection of nectar and/or pollen. Accessed from https://www.ars.usda.gov/ARSUserFiles/OPMP/Attractiveness%20of%20Agriculture%20Crops%20to%20Pollinating%20Bees%20Report-FINAL_web%20version_Jan%202018.pdf (2017).

52. Wheelock, M. J. & O'Neal, M. E. Insect pollinators in Iowa cornfields: community identification and trapping method analysis. PLoS One 11, e0143479, https://doi.org/10.1371/journal.pone.0143479 (2016).

53. Wheelock, M. J., Rey, K. P. & O’Neal, M. E. Defining the insect pollinator community found in Iowa corn and soybean fields: implications for pollinator conservation. Environmental Entomology 4, 1093–1106, https://doi.org/10.1093/ee/nvw1087 (2016).

54. Gathmann, A. & Tschirnke, T. Foraging ranges of solitary bees. Journal of Animal Ecology 71, 757–764, https://doi.org/10.1046/j.1365-2656.2002.00641.x (2002).

55. Richards, M. H. et al. Bee diversity in naturalizing patches of Carolinian grasslands in southern Ontario, Canada. Canadian Entomologist 143, 279–299, https://doi.org/10.4039/n11-010 (2011).

56. MOECC. Corn and soybean neonicotinoid-treated seed data. Accessed from https://www.canada.ca/data/corn-and-soybean-neonicotinoid-treated-seed-data (2017).

57. OMAFRA. Neonicotinoid regulations. Accessed from https://www.ontario.ca/page/neonicotinoid-regulations (2015).

58. Beekman, M. & Ratnieks, F. L. W. Long-range foraging by the honey-bee, Apis mellifera. Functional Ecology 14, 490–496, https://doi.org/10.1046/j.1365-2435.2000.00443.x (2000).
70. R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical
71. Delignette-Muller, M. L., Pouillot, R., Denis, J-B. & Dutang, C. Fitdistrplus: help to fit of a parametric distribution to non-censored
72. Dinter, A., Brugger, K. E., Frost, N.-M. & Woodward, M. D. Chlorantraniliprole (Rynaxypyr): a novel DuPont insecticide with low
73. Ruzhong, G., Rui, C. & Liangyan, C. Evaluation on toxicity and safety of imidacloprid to environmental organisms. Pesticide Science and Administration 20(3) (1999).
74. Stark, J. D., Jepson, P. C. & Mayer, D. F. Limitation to use of topicality data for prediction of pesticide side effect in the field. Journal of Economic Entomology 88, 1081–1088, https://doi.org/10.1093/jee/88.5.1081 (1995).
75. EFSA. Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid considering the uses as seed treatments and granules. EFSA Journal 16, 5178, https://doi.org/10.2903/j.efsa.2018.5178 (2018).
76. Dinter, A., Brugger, K. E., Frost, N.-M. & Woodward, M. D. Chlorantraniliprole (Rynaxypyr): a novel DuPont insecticide with low toxicity and low risk for honey bees (Apis mellifera) and bumble bees (Bombus terrestris) providing excellent tools for uses in integrated pest management. Julius-Kühn-Archives 423, 84–96 (2009).
77. Delignette-Muller, M. L., Pouillot, R., Denis, J-B. & Dutang, C. Fitdistrplus: help to fit of a parametric distribution to non-censored or censored data. R package version 0.1-3, https://CRAN.R-project.org/package=fitdistrplus (2010).
78. R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, http://www.r-project.org/ (2008).

Acknowledgements
We would like to thank all 18 Ontario farmers who allowed us access to their land and crops, Beatrice Chan and Katie Fisher for their assistance with field sampling, Linda Lissemore for advice on residue analyses, Elaine Roddy for providing input into production practices and pesticide use patterns on farms, providing farm contacts, and sampling on all the farms in southwestern Ontario, and Jim Chaput for technical advice on pesticide registration and use in Cucurbita crops. This work was supported by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) grant UofG2015-2466 (awarded to N.E.R. and D.S.W.C.), the Ontario Ministry of Environment and Climate Change (MOECC) Best in Science grant BIS201617-06 (awarded to N.E.R.), Natural Sciences and Engineering Research Council (NSERC) Discovery grants 2015-06783 and 2018-04641 (awarded to N.E.R. and R.S.P. respectively), the Fresh Vegetable Growers of Ontario (FVGO) awarded to N.E.R. and D.S.W.C., and the Food from Thought: Agricultural Systems for a Healthy Planet Initiative, by the Canada First Research Excellence Fund (grant 000054). D.S.W.C. was supported by the George and Lois Whetham Scholarship in Food Systems, an Ontario Graduate Fellowship, the Keith and June Laver Scholarship in Horticulture, the Fred W. Presant Scholarship, and a Latournelle Travel Scholarship. N.E.R. is supported as the Rebanks Family Chair in Pollinator Conservation by The W. Garfield Weston Foundation.

Author Contributions
D.S.W.C., R.S.P. and N.E.R. conceived and designed the project. D.S.W.C. carried out the experimental work and collated MOECC datasets. D.S.W.C., R.S.P. and J.L.R.-G. carried out the probabilistic risk assessments and statistical analyses. All authors contributed to writing the paper.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-47805-1.

Competing Interests: The authors declare no competing interests.

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