Risks of exposure to systemic insecticides in agricultural soil in Ontario, Canada for the hoary squash bee (*Peponapis pruinosa*) and other ground-nesting bee species

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Abstract

Insecticide exposure is an environmental factor of concern relating to pollinator health. Recent increases in use of systemic insecticides, particularly neonicotinoids, have led to considerable research into the potential impacts for bees of exposure to these insecticide residues via the nectar and pollen of treated crops or contaminated wild flowers. However, although the majority of bee species are ground-nesting, the risk of exposure to insecticides in soil has not yet been evaluated. Here we use the hoary squash bee (*Peponapis pruinosa*) as a model system to provide the first evaluation of the risk of exposure to insecticide residues in soil to ground-nesting solitary bee species. The evaluation assessed agrochemical residues from the nectar, pollen and soil from Cucurbita-crops (e.g. squash and pumpkin) grown in southern Ontario in 2016, and soil insecticide residue data collected from field crops by the Ontario government. Systemic insecticide residues were rarely detected in the nectar and pollen of Cucurbita-crops in 2016, and hazard assessment deemed these potential routes of exposure to be non-hazardous for honey bee lethal dose endpoints. In contrast, quantifiable pesticide residues were frequently detected in soil suggesting this route of exposure to be hazardous for honey bee lethal dose endpoints, leading to further assessment using probabilistic risk assessments. Concentrations of clothianidin, imidacloprid, thiamethoxam (neonicotinoid insecticides) and chlorantraniliprole (an anthranilic diamide insecticide) in soil samples were plotted to produce an environmental exposure distribution for each insecticide. A honey bee LC₅₀ and a solitary bee LC₅₀ endpoint were converted to exposure endpoints using the amount of soil excavated by hoary squash bees during nest construction, for both acute and chronic exposure scenarios. The probability of exceedance of each exposure endpoint was calculated and compared to an acceptable risk threshold (i.e. 5% exceedance). In the acute exposure scenario, risk to hoary squash bees was below acceptable
threshold levels for all residues evaluated using the honey bee LC$_{50}$, but exceeded the threshold for clothianidin and imidacloprid using the solitary bee LC$_{50}$. In the chronic exposure scenario, risk from exposure to clothianidin and imidacloprid exceeded the threshold for both the honey bee and solitary bee LC$_{50}$s, and for chlorantraniliprole risk only exceeded the threshold for the solitary bee LC$_{50}$. Using the hoary squash bee as a model, the wider implications for other ground-nesting bees that pollinate crops was explored using insecticide residues from soil samples taken from Ontario field crops. Probabilistic risk assessments suggest that risk to ground-nesting bees is high from clothianidin residues in soils, even when exposure is acute, and substantial for both thiamethoxam and imidacloprid under chronic exposure scenarios. These results demonstrate the urgent need to consider direct exposure to pesticides in soil for ground-nesting bees as part of risk assessments and provides a potential model for evaluating risk from this type of exposure.

Keywords: crop pollination; environmental exposure distribution; insect pollinators; neonicotinoid insecticide; probabilistic risk assessment; solitary bees; systemic pesticides

**Introduction**

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 provide (Calderone 2012; Vanbergen et al. 2013). For bee populations living in proximity to agricultural production, exposure to pesticides is one of the major environmental stressors likely affecting population health (Krupke et al. 2012; Vanbergen et al. 2013).

*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 to mediate pollination (Whitaker 1962). *Cucurbita* crops grown in Ontario have several economically important pests, the most serious of which is the cucumber beetle (*Acalymma vittatum*), a vector of bacterial wilt (*Erwinia tracheiphila*) (OMAFRA 2009). 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 the health of bee populations for the essential pollination services they provide.
In Ontario, three neonicotinoids (imidacloprid, clothianidin, thiamethoxam) are commonly used in *Cucurbita*-crop production to control insect pests (OMAFRA 2014). Although they are highly effective against pests, neonicotinoid insecticides are of environmental concern because of their relatively high toxicity to insects, their systemic nature, their persistence, and their extensive use in agriculture (Jeschke *et al.* 2011; Goulson 2013). In agriculture globally, about 60% of neonicotinoids are applied as seed coatings or as in-furrow soil applications, with the remaining applied as foliar sprays (Jeschke *et al.* 2011). Neonicotinoid residues have been found in agricultural soil (Krupke *et al.* 2012; Goulson 2013) and in the nectar and pollen of *Cucurbita* flowers (Dively & Kamal 2012; Stoner & Eitzer 2012). Neonicotinoids have also been found to persist in soil resulting in their detection in agricultural soils in the season following their application (Goulson 2013, Jones *et al.* 2014 - Britain; MOECC 2016 - Canada).

Although little is known about their impacts on most wild bees, the impacts of exposure to neonicotinoids on managed bees are well documented and include sublethal effects at subcellular to population levels, among eusocial and solitary bees, and at both adult and larval stages (Godfray *et al.* 2014, 2015; Alkassab & Kirchner 2017).

Possible routes of insecticide exposure for bees including oral exposure via ingestion of nectar, pollen, aphid honeydew, guttation water, chemigation water from soil treatments and contact exposure from abraded dust, sprays, microencapsulated particles, guttation water, and chemigation water from soil treatments (Godfray *et al.* 2014, 2015; Boyle *et al.* 2018). For non-*Apis* bees, all developmental stages (adult and larvae) may be exposed to pesticide residues in nectar and pollen, though the extent of exposure is likely greatest for adult females because they consume pollen and nectar during sexual maturation and egg laying (Michener 2007; Cane 2016; Sgolastra *et al.* 2018), consume nectar to fuel their foraging flights and nest building activities throughout their lives, and handle both pollen and nectar to provide food for their offspring (Mathewson 1968; Cane 2016). Males consume nectar and pollen during sexual maturation and nectar thereafter to fuel flight (Michener 2007). Larval stages consume pollen and nectar in their larval provisions and contact those provisions topically within their nest cells because the they lie directly on their provisions as they consume them (Michener 2007). For non-*Apis* bees, exposure may also be via nesting sites and nesting materials (Fisher & Moriarty 2011; Sgolastra *et al.* 2018). For ground-nesting bees, exposure from nesting sites is via soil contacted during nest excavation and construction for adult females, and via contact with the soil that forms nest cells.
during larval development. However, the nest cells of many ground-nesting bee species have a water-resistant coating that may preclude exposure to pesticide residues in soil for larvae (Michener 2007). As adult male ground-nesting bees do not participate in nest construction (Michener 2007), they are not expected to be substantially exposed to pesticide residues in soil. The persistence of neonicotinoid residues in soil generates the potential for both acute and chronic contact exposure for ground-nesting bees. The hydrophilic properties of this class of insecticides mean that they do not easily penetrate the intact cuticle of insects, and therefore that lethal contact exposures to neonicotinoids are typically higher, and show much less variability among bee species, than lethal oral exposures (Decourtye & Devillers 2012). Although no published data quantifying the extent of neonicotinoid uptake from soil by ground-nesting bees exist, the translocation of neonicotinoids to bees from residues in dust during corn planting is well documented (Girolami et al. 2013), and may be somewhat representative of translocation of neonicotinoids from soil to bees that excavate ground nests.

In eastern North America, the hoary squash bee (*Peponapis pruinosa*) is among the most important pollinators of *Cucurbita* crops, along with honey bees (*Apis mellifera*), and bumble bees (*Bombus* spp.) (Willis & Kevan 1995; Artz et al. 2011). Hoary squash bees are solitary bees that excavate soil to build their nests in the ground (Figure 1) within and around agricultural fields where *Cucurbita* crops are grown (Willis 1991). They are oligolectic, consuming a diet consisting almost entirely of *Cucurbita* pollen and nectar (Hurd et al. 1974). Because they have no wild host plants in eastern North America, they are obligately associated with *Cucurbita* crops (Lopez-Uribe et al. 2016). In 2014, in recognition of the unique risk to hoary squash bees from exposure to neonicotinoids, the Pest Management Regulatory Agency (PMRA) of Health Canada initiated a special review of registered neonicotinoid insecticides used on cucurbit crops (PMRA 2014). Because the hoary squash bee is common, nests in large noticeable aggregations, and has a well-documented natural history (Mathewson 1968; Hurd et al. 1974; Willis 1991; Willis & Kevan 1995), this species is a good candidate to be a model species for evaluating risk from exposure to pesticides in Ontario agricultural soils for other less-studied, more elusive ground-nesting solitary bee species.

Substantial knowledge gaps remain around the toxicity and effects of neonicotinoids encountered by arthropods in soil, including bees that excavate nests and undergo development in the ground. This study is the first to address the potential risk of soil as a route of direct exposure to
insecticides for ground-nesting bees. The aims of this study are (1) to evaluate which insecticides pose a potential hazard to hoary squash bees; (2) to evaluate which exposure matrices (soil, pollen, and nectar) pose the greatest potential hazard to hoary squash bees; (3) to determine which hoary squash bee developmental stage (adult female or larvae) is at greatest hazard; and (4) to evaluate risk for all ground-nesting solitary bees on farms using the exposure profile of hoary squash bees as a surrogate. We anticipate that data from this study will contribute to risk assessments for hoary squash bees and other solitary bees.

Methods

Sampling

In July and August 2016, the period during which adult female hoary squash bees are building nests and foraging on Cucurbita crops, 29 samples of soil, nectar and pollen were taken from 18 farms across southern Ontario (11 farms were sampled twice, and 7 farms were sampled once). Potential for cross contamination of samples was minimized by using disposable gloves, single-use containers and instruments, and thorough cleaning of the soil corer between farms. Soil samples were taken from fields where Cucurbita crops were growing as this is often where hoary squash bees construct their nests. Using a soil corer, ten soil samples were taken per field from the top 15 cm of soil. The ten core samples were combined and subsampled to produce a single 3 g sample for residue analysis.

To maximize the amount of pollen and nectar available for sampling, and to prevent contamination from flower visitors, nectar and pollen samples were collected directly from staminate (male) flowers from which insects had been excluded. Nectar was harvested from 25 staminate flowers into a single 2 mL micro-centrifuge tube using a 20 µL micro-pipette until the micro-centrifuge tube was full. To collect pollen, synandria from the same 25 staminate flowers were excised, and the pollen from all 25 synandria was dislodged into a single-use plastic container using a disposable knife and weighed.

To determine the maximum number of pollen grains per synandrium, forty synandria with full pollen were gathered individually into 2 mL microcentrifuge tubes to which 0.5 mL of 70% alcohol was added. In the lab, pollen was dislodged from synandria by centrifuging at 2500 rpm for 3 minutes. The stripped synandria were then removed and spot checked to confirm that the
method effectively removed all pollen. Subsequently, the microcentrifuge tubes were topped up with 50% glycerin solution to bring the total volume of liquid in the tubes to 2 mL. The number of pollen grains in a full synandrium was calculated by averaging the number of pollen grains counted in five 5-µL aliquots and relating this back to the full 2 mL volume. Before removing aliquots, the pollen was thoroughly distributed in the glycerin solution by mixing with a mini vortex mixer.

**Residue Analysis**
All soil, pollen, and nectar samples collected from farms were submitted for analysis to University of Guelph Agri-Food Laboratories (ISO/IEC 17025 accredited). Samples were analyzed using their TOPS-142 LC pesticide screen, modified from the Canadian Food Inspection Agency (CFIA) PMR-006-V1.0 method. Pesticides were extracted using the QuEChERS Method (Schenck and Hobbs 2004). Extracts were analyzed using high performance liquid chromatography paired with electrospray ionization and tandem mass spectrometry and gas chromatography paired with tandem mass spectrometry. Limits of detection and quantification for all residues detected in samples of soil, pollen, and nectar by the University of Guelph Agri-Food Laboratories are summarized in Table 3.

**Extent of Exposure**
Information from the literature and this study were used to determine the realistic amount of pollen, nectar, and soil that hoary squash bees would be exposed to via contact or ingestion. This study examined exposure amounts for adult female and larval squash bees only, as information is lacking about exposure in male hoary squash bees. However, it is reasonable to expect that males are less exposed to pollen and nectar than females because they do not provision nest cells. Males are not exposed to soil during this period of the lifecycle as they do not participate in nest construction (Mathewson 1968).

In this study, the mass of a single *Cucurbita* pollen grain was determined by dividing the mean mass of pollen grains per synandrium (mean = 0.0302 g; n = 25) by the mean number of pollen grains per synandrium (mean = 18,438, n = 40)(0.0302 g / 18,438 pollen grains = 1.64 x 10^-6 g/pollen grain). For larvae, the amount of pollen consumed (0.0468 g pollen/nest cell) was calculated by multiplying by the mass of a single pollen grain (as calculated above = 1.64 x 10^-6 g/pollen grain).
g/pollen grain) by the mean number of pollen grains in hoary squash bee larval provisions (i.e., 28,543 pollen grains: Willis 1991). Larval exposure to pesticide residues via cuticular contact with pollen in provisions was not evaluated because of lack of information on the proportion of pesticide transferred across the nest lining, and contact toxicity of pesticides to larvae.

Contact exposure for adult females was assessed to be five times that for each larva because each female provisions five cells within a nest (Mathewson 1968). The amount of pollen ingested by adult females could not be calculated because information on their nutritional demands is lacking. Currently no data exist regarding the nectar requirements of either adult female or larval hoary squash bees. However, pollen-collecting honey bee workers are most like adult female hoary squash bees in their foraging behaviour. For honey bees, pollen-collecting foraging workers have been estimated to consume 10.4 mg sugar/day to fly between their nest to forage patches and from flower-to-flower within those patches (Rortais et al. 2008). Based on this assumption, and that pumpkin (Cucurbita pepo) nectar contains 40% sugar (Willis 1991), female hoary squash bees that forage during a 30-day period, would need to consume approximately 312 mg sugar in 780 mg of Cucurbita nectar to meet their lifetime energy requirements. This is likely an overestimation of nectar consumption for female hoary squash bees as the foraging radius of squash bees from nest to flower patch is much smaller than that of honey bees (honey bee foraging radius: mean = 5.5, maximum = 15 km (Beekman & Ratnieks 2000); oligolectic solitary bee foraging radius is < 260 m (Gathmann & Tscharnke 2002)).

Using hoary squash bee nest dimensions from Mathewson (1968), the volume of soil excavated by a female squash bee to build a nest is 25.19 cm³ (Table 1). This can be multiplied by the bulk density (BD) of loam soil, a type common in agricultural soils (BD_loam = 1.33 g/cm³: USDA 2008), to calculate the soil mass to which a female hoary squash bee is exposed (25.19 cm³ x 1.33 g/cm³ = 33.51 g). This mass represents the total amount of soil to which a female hoary squash bee is exposed cumulatively during construction of a single nest over 30 days of activity. Acute (48 h) exposure was calculated by dividing cumulative exposure by 15 (33.5g / 15 = 2.23 g). A summary of exposure routes and extent of exposure for the hoary squash bee are provided in Table 2.
Effect Endpoints

Effect endpoints (e.g., the concentration causing a 50% reduction in survival, LC₅₀) are required in a risk assessment to characterize the effect of a chemical. Unfortunately, effect endpoints are not currently known for the hoary squash bee at any developmental stage for any pesticide, which represents a considerable knowledge gap for this agriculturally-relevant species. For this hazard assessment, the adult honey bee LD₅₀ for oral exposure to nectar or pollen or the adult honey bee LC₅₀ for contact exposure to pollen or soil was used. Honey bee values were used because current regulatory standards consider the honey bee to be an adequate proxy for all bee species (US-EPA 2014), and lethal doses for larval stages are rarely available (Godfray et al. 2014; Alkassab & Kirchner 2017). Honey bee LD₅₀ and LC₅₀ values were obtained from the US-EPA Pesticide Ecotoxicity Database of the Office of Pesticide Programs, Ecological Fate and Effects Division, of the U.S. Environmental Protection Agency (database: http://www.ipmcenters.org/Ecotox/) and published literature. Multiple LC₅₀ values were available for clothianidin, imidacloroprid, and thiamethoxam. Consequently, the geometric mean of these lethal doses, reported from seven sources (US-EPA database; Iwasa et al. 2004; European Commission EU pesticides database; Sanchez-Bayo & Goka 2014; Ruzhong et al. 1991; Stark et al. 1995; Goulson et al. 2008) were used in this study (Table 4). For adult female hoary squash bees, contact honey bee LC₅₀ values were used for soil and pollen as exposure to these matrices would be via contact, rather than ingestion. Oral honey bee LD₅₀ values were used for adult female hoary squash bee ingestion of nectar, and larval hoary squash bee ingestion of pollen.

For the probabilistic risk assessment in this study, which focused on soil exposure, the effect endpoints relevant to solitary bees include: (1) the geometric mean honey bee contact LC₅₀ endpoint, (2) the lowest reported honey bee contact LC₅₀ endpoint in the literature (Iwasa et al. 2004; US-EPA database), (3) surrogate solitary bee contact lethal endpoints (honeybee LC₅₀/10; Arena & Sgolastra 2014; EFSA 2018), and (4) the concentration causing no observable adverse effect (NOAEC) endpoint from contact with clothianidin for honey bees (1.25 ng/bee; Lambin et al. 2001). No NOAEC was available for the active ingredients (a.i.) imidacloroprid, thiamethoxam, or chlorantraniliprole. For clothianidin, imidacloroprid, thiamethoxam, and chlorantraniliprole, contact effect endpoints were converted to soil-exposure endpoints as follows:

\[
\text{Exposure Endpoint (ng a.i./g matrix)} = \frac{\text{Effect Endpoint (ng a.i./bee)}}{\text{Amount of soil exposure (g matrix/bee)}}
\]
Hazard Quotients

The hazard quotient (HQ) was calculated for each residue detected in each exposure matrix as follows:

\[
\text{Hazard Quotient} = \frac{\text{(Concentration of residue in matrix)} \times \text{(Amount of exposure to matrix)}}{\text{Honey bee LD}_{50} \text{ or } LC_{50}}
\]

A considerable hazard to hoary squash bee populations because exposure exceeds the honey bee lethal dose. In this hazard assessment, the concentration of residues was calculated by taking the geometric mean of all quantifiable concentrations in samples in the study for each matrix, ignoring all samples that were below the limit of detection (LOD). Concentrations of residue in each matrix were reported in ng a.i./g matrix, amount of exposure to the given matrix was reported in g matrix/bee, and LD50s or LC50s are reported in ng a.i./bee.

Hazard quotients were summed for each type of pesticide (fungicide or insecticide) and exposure matrix (soil, pollen, nectar) to determine which pesticide type and matrix were potentially most hazardous to hoary squash bees. Hazard quotients were also summed for adult females and larvae to determine which developmental stage faced the greatest hazard.

Environmental Exposure Distributions

Distributions

To evaluate the risk to ground-nesting bees from exposure to soil during nest excavation and construction, empirical environmental exposure distributions (EEDs) were generated from the concentration of insecticides measured in soil by fitting the data to a log-normal or gamma distribution via maximum likelihood estimation (MLE), using the fitdistrcens function in the R package fitdistrplus (Delignette-Muller et al. 2010; R Development Core Team 2008). This function allows for the fit of censored (right-, left-, or interval-censored) data enabling the use of the available “non-detect”, and interval (LOD-LOQ) data. The fitdistrplus function calculates the probability plotting position using Hazen’s rule, with probability points of the empirical distribution calculated as \((1:n - 0.5)/n\), where n is the total number of data points (Delignette-Muller et al. 2010). Interval data (non-detects, LOD-LOQ) were ranked according to the midpoint of the interval. Confidence intervals (95%) for the distribution parameters and distribution estimates were calculated via nonparametric bootstrapping (1000 iterations) with the bootdiscens function in the fitdistrplus package. The fit to other distributions (Weibull and
Exponential) were also tested, and the best fit was chosen via comparison of the Akaike information criterion (AIC supported by visual inspection of the fit to the actual data). The gamma distribution provided the best fit to construct EEDs for clothianidin and chlorantraniliprole measured in soil, and the log-normal distribution provided the best fit for imidacloprid and thiamethoxam. The percent rank was subtracted from 100 to determine the percent exceedance. For clothianidin, imidacloprid, thiamethoxam, and chlorantraniliprole EEDs, exposure endpoints (Table S1), exceedances (Table S2), and model parameters (Table S3) are listed in supplementary information.

The exposure endpoints described (geomean LC₅₀, least LC₅₀, solitary bee surrogate LC₅₀, NOAEC) were used to calculate the percent exceedance of the EEDs. In this study, percent exceedance is the probability that hoary squash bee adult females will be exposed to insecticide residues at concentrations higher than the pre-determined exposure endpoints. For the purposes of this assessment, an exceedance value lower than 5% was assigned as an acceptable risk for this species because it assures protection 95% of the time.

Exposure Scenarios

Chronic and Acute

Exposure endpoints for the soil matrix were calculated for both acute and chronic exposure scenarios. In the chronic exposure scenario, exposure was to the total amount of soil that an adult female excavates during the construction of a single nest, typically containing 5 nest cells and constructed during a 30-day period (Mathewson 1968; Table 1). For the acute exposure scenario, exposure was the total amount of soil that adult females excavate in a 48-hour period, calculated by dividing the chronic exposure amount of soil by 15. The chronic exposure level was 33.5 g of soil, and the acute soil exposure amount was 2.23 g.

Environmental exposure distributions (EEDs) were constructed for each neonicotinoid for chronic exposure to soil (30 days, 33.5 g soil) because the effects of neonicotinoids on the nicotinic acetylcholine receptors (nACHRs) of insects are cumulative and irreversible (Tennekes & Sanchez-Bayo 2011) and neonicotinoids have been shown to be relatively persistent in soil (Jones et al. 2014 – Britain; MOECC 2016 – Canada). EEDs for an acute exposure scenario (48 h, 2.23 g soil) for neonicotinoid residues in soil were also constructed. EEDs for chlorantraniliprole were only estimated for acute exposure as existing evidence from honey bees
suggests their effects may be transient (Dinter et al. 2009). Honey bees dosed with chlorantraniliprole, orally or topically under artificial test conditions, became lethargic but recovered within 48-72 hours after exposure (Dinter et al. 2009).

**Cucurbita** and Field Crop Soils

To compare exposure of hoary squash bees on *Cucurbita*-crop farms, and to expand the scope of this assessment to all solitary ground-nesting bees that nest in agricultural soil, EEDs were created using data from both the *Cucurbita*-crop farms sampled in this study and from a publicly-available dataset provided by the Ontario Ministry of the Environment and Climate Change (MOECC 2016). These MOECC data reported neonicotinoid residues in soil from 38 agricultural sites in southern Ontario in 2016. Only data from soil samples taken at a depth of 15 cm prior to seeding or pesticide application in the spring were used from this MOECC data set. The limit of detection reported for the MOECC data set was 0.05 ng/g for clothianidin, imidacloprid, and thiamethoxam.

Insecticide residues in the samples taken as part of this study represent biologically-relevant exposure specifically for the hoary squash bee on *Cucurbita* farms. Neonicotinoid residues in the MOECC samples represent the “background” levels of neonicotinoids in Ontario agricultural soils that persist from a previous cropping cycle, rather than concentrations that are detected after neonicotinoids are applied within the current cropping cycle. Therefore, MOECC data levels represent the minimum exposure to neonicotinoids for ground-nesting bees in soil within an Ontario agricultural context. In reality these bees could experience greater exposure if they are nesting in soil after neonicotinoids are applied in the current cropping cycle.

Hoary squash bee exposure to soil during nest excavation was used as a surrogate for exposure in all ground-nesting solitary bee species. Although using squash bee exposure as a surrogate for other ground-nesting bee species is not a perfect model, it is a starting point to understand risk from insecticide residues in soil for ground-nesting solitary bees. For ground-nesting solitary bees, tunnel diameter is related to bee size (because bees excavate tunnels and nest cells large enough for themselves: Michener 2007). However, bee body size does not necessarily appear to be correlated to the depth that vertical tunnels in nests are excavated in soil (Cane 1991). Solitary bees are physiologically limited in their reproductive capacity and generally build 1-8 brood cells per nest (Michener 2007). 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 basis upon which to compare exposure for different solitary bee species in the future. Although smaller bees are generally more sensitive to insecticide residues (Devillers et al. 2003), they will handle less soil (and be less exposed) than larger bees because the diameter of their nest tunnels and cells are smaller.

**Results**

**Pesticides in Cucurbita-farm Samples**

Residues of 7 insecticides, 6 fungicides, and 2 herbicides were detected in samples taken from farms growing *Cucurbita* crops. The list of pesticides detected, including information about limits of detection and quantification (LOD/LOQ), frequency of detection, and mean and maximum concentrations in each exposure matrix are presented in Table 3. Herbicide residues were not assessed further in this study as resulting hazard quotients were extremely low, which is not surprising considering that herbicides are targeted to remove plants rather than insects.

The three exposure matrices (pollen, nectar, soil) show different frequencies of detection and pesticide residue profiles (Figure 2; Table 3). Soil was the most contaminated with 5/7 insecticides and 5/6 fungicides detected. Pollen contained residues of 4/7 insecticides and residues of all 6 fungicides, but maximum residue concentrations were lower, and residues were detected much less frequently, than in soil - despite generally lower LODs for nectar and pollen than soil. Nectar was the least contaminated exposure matrix with only 3/7 insecticides and 3/6 fungicides detected, with the lowest residue concentrations and lowest frequency of detection. However, imidacloprid was found with the same frequency in both nectar and pollen, and was the only insecticide detected in all three matrices. Imidacloprid was detected in 21% of soil samples, 3% of pollen samples and 3% of nectar samples. Thiamethoxam was present only in a single soil sample. Clothianidin was detected in 34% of soil samples, but was not detected in either nectar or pollen. The insecticides chlorantraniliprole and carbaryl were detected in soil samples (24% and 10% respectively) and pollen samples (3% and 7% respectively), but were not detected in nectar. The insecticides methomyl and dimethoate were not detected in soil, but dimethoate was detected infrequently in pollen and nectar (both 3%), and methomyl was detected 7% of nectar samples. The fungicide picoxystrobin was also detected infrequently in nectar (3%) and pollen (10%). The fungicides pyraclostrobin, and propamocarb were detected in
all three matrices, while boscalid, quinoxyfen and difenoconazole were detected in soil and pollen, but not in nectar.

**Hazard Assessment**

Pesticides

Hazard quotients for fungicide and insecticide residues are presented for each exposure matrix (soil, pollen, nectar) for either adult females or larvae as appropriate in Table 4. As expected, insecticides exhibited a considerably higher combined hazard quotient (HQ\text{insecticide} = 15.11) across all exposure matrices than fungicides (HQ\text{fungicide} = 0.03: Table 4). Among the insecticides detected, only the neonicotinoids clothianidin, imidacloprid, and thiamethoxam, had HQs ≥ 1 in soil, while the combined HQ of all non-neonicotinoid insecticides was less than 1 in the same matrix (HQ\text{non-neonicotinoid} = 0.15: Table 4). However, although chlorantraniliprole had an HQ < 1, it was included in further probabilistic risk assessment for comparative purposes because it was found in 24% of samples and is also systemic insecticide. The physical, chemical and environmental fate properties of imidacloprid, clothianidin, thiamethoxam and chlorantraniliprole are summarized in Table 5.

Exposure Matrices

HQ values for pollen, including both adult and larval exposure (HQ = 0.36), and nectar were low (HQ = 0.18) relative to values for soil (Table 4). However, only adult female exposure was assessed for nectar as no information exists in the literature about the amount of nectar consumed by larvae or adult males. Chlorantraniliprole, a newer systemic insecticide that is being used within *Cucurbita* crop systems in Ontario, was detected in only one pollen sample and not found in nectar samples. Imidacloprid was also detected in one pollen sample and 6.9% of nectar samples; other neonicotinoids were not detected in these matrices. Low hazard quotients for pollen and nectar in this study reflect the low concentrations of all the insecticides detected in these exposure matrices. Imidacloprid was the largest contributor to the combined HQs for these matrices, with a contribution of 89% and 99% of hazard in pollen and nectar, respectively (Table 4). As HQs for pollen and nectar were < 1, no further assessment of risk was deemed necessary for these exposure matrices.
The combined HQ for insecticides in soil was very high (HQ_{soil} = 14.56) relative to nectar and pollen (Table 4). The soil HQ was mostly attributable to the neonicotinoid residues (HQ_{imidacloprid} = 2.65, HQ_{clothianidin} = 1.93, HQ_{thiamethoxam} = 9.41: Table 4). However, the HQ calculation for thiamethoxam is less reliable as it is based on a single sample containing detectable residues. This underlines the need to assess risk using probabilistic methods.

**Developmental Stage**

There appears to be a much greater hazard to adult females (HQ_{adult female} = 15.05 including thiamethoxam; HQ_{adult female} = 5.03 excluding thiamethoxam) relative to larval hoary squash bees (HQ_{larvae} = 0.06), mostly because of their exposure to neonicotinoid residues in soil during nest construction (HQ_{soil} = 13.99 including thiamethoxam; HQ_{soil} = 4.58 excluding thiamethoxam: Table 4).

**Probabilistic Risk Assessment**

**Hoary Squash Bees in Cucurbita Crop System**

Environmental exposure distributions (EEDs) for the acute exposure scenario (exposure to 2.23 g soil in 48 hours), indicate that clothianidin soil residues showed exceedance below 5% based on the geometric mean honey bee LC_{50} and the lowest honey bee LC_{50} endpoints. However, the exceedance for the solitary bee surrogate lethal dose endpoint was high (28.3%), as was exceedance for the honey bee NOAEC endpoint (11.4%; Figure 3A, Table S2). Imidacloprid showed exceedance below 5% for the geometric mean honey bee LC_{50} (3.5%) and exceedances greater than 5% for both the lowest honey bee LC_{50} (8.9%) and the solitary bee surrogate LC_{50} (31.2%; Figure 3B, Table S2). In the acute exposure scenario, Chlorantraniliprole did not exceed 5% for any of the effect endpoints (Table S2) and so appears to pose no risk.

For the chronic exposure scenario (exposure to 33.5 g soil over 30 days), exceedance for clothianidin was relatively high for all soil exposure endpoints in Cucurbita growing systems (Figure 4A, Table S2). The geometric mean honey bee LC_{50}s resulted in a 35.8% exceedance, with increasing exceedances for the lowest honey bee LC_{50} (44.3%), the honey bee NOAEC (57.1%) and the solitary bee surrogate lethal dose (68.7%; Figure 4A, Table S2). For imidacloprid, the exceedance for all exposure endpoints was also high under the chronic exposure scenario, at 39.8% for the geometric mean honey bee LC_{50}s, 57.8% for the lowest
honey bee LC$_{50}$, and 85.4% for the solitary bee surrogate LC$_{50}$ (Figure 4B, Table S2). Exceedance under the chronic exposure scenario for chlorantraniliprole was below the acceptable range of 5% for the geometric mean honey bee LC$_{50}$ and lowest honey bee LC$_{50}$ (Figure 4C, Table S2). However, for the solitary bee surrogate LC$_{50}$, exceedance was 11.9%, more than double the acceptable exposure probability (Figure 4C, Table S2). Because of high limits of detection and quantification (LOD/LOQ) for thiamethoxam rendered only a single quantifiable residue sample, EEDs could not be reliably fitted to data for this active insecticide for either exposure scenario.

All Ground-Nesting Bees in Field Crop System
Based on publicly-available data (MOECC 2016) for Ontario’s agricultural soils, 96.34% of soil samples (n = 82) had detectable clothianidin residues, 10.97% of soil samples had detectable imidacloprid residues, and 81.48% had detectable thiamethoxam residues. For both the acute and chronic exposure scenarios for all ground-nesting solitary bees in Ontario’s agricultural soils, EEDs were constructed using hoary squash bee exposure amounts (2.23 g soil-acute; 33.5 g soil-chronic) as a surrogate.

In the acute exposure scenario, exceedance was only greater than the 5% threshold for the solitary bee surrogate LC$_{50}$ endpoint when considering EEDs for either imidacloprid (Figure S1) or thiamethoxam (Figure 5B). In contrast, the probability of exceedance was greater than 5% for all exposure endpoints for clothianidin (honey bee geometric mean LC$_{50}$: 11.72%; honey bee least LC$_{50}$: 27.25%; NOAEC: 57.88%; solitary bee surrogate LC$_{50}$: 81.85%), suggesting that risk to ground-nesting bees is high from clothianidin in soils, even when exposure is acute (Figure 5A).

The probability of exceedance for clothianidin in the chronic scenario was very high for all exposure endpoints (honey bee geometric mean LC$_{50}$: 87.68%; least honey bee LC$_{50}$: 92.4%; NOAEC: 96.76%; solitary bee surrogate LC$_{50}$ t: 98.82%; Figure 6A). For thiamethoxam, probability of exceedance was lower (honey bee geometric mean LC$_{50}$: 35.7%; honey bee least LC$_{50}$: 37.36%; solitary bee surrogate LC$_{50}$: 78.42%) but still greatly exceeded the 5% acceptable exceedance threshold (Figure 6B). Probabilities of exceedance for imidacloprid under the chronic exposure scenario was below 5% for the honey bee geometric mean LC$_{50}$ endpoint.
(4.16%) and slightly exceeded this threshold for the honey bee least LC$_{50}$ endpoint (5.89%) and the solitary bee surrogate LC$_{50}$ endpoint (9.24%) (Figure S2; Table S2).

**Discussion**

The differences in the residue profiles of the exposure matrices (soil, pollen, nectar) in *Cucurbita* growing systems in Ontario (Figure 2) are related to the chemical and physical properties of the pesticides (Table 5), the application method (seed coating, direct soil application, or foliar spray) and timing of application. Comparing the three matrices, soil had the greatest number of neonicotinoids detected (clothianidin, imidacloprid and thiamethoxam present in a single sample), while 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). Analyses of the nectar and pollen of *Cucurbita* crops in the United States have reported higher mean imidacloprid residues 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: Dively & Kamel 2012). These differences could be because samples in this study were taken 8 weeks after pesticide application corresponding to the period of *Cucurbita* flowering and hoary squash bee activity in Ontario compared to samples taken 5 weeks after application by Dively and Kamel (2012). Stoner and Eitzer (2012) reported higher concentrations of imidacloprid and thiamethoxam in *Cucurbita* crops after application to soil than found in this study, which may be because they included anther and nectary tissue in their analysis of residues in pollen and nectar.

**Hazard Assessment**

It is important to highlight that calculations in the analysis of the hazard of pesticides to the hoary squash bee within *Cucurbita* crops in Ontario are based on lethal doses for adult honey bees, as this species is presently the regulatory standard for testing toxicity in bees (US-EPA 2014). However not all bee species (Arena & Sgolastra 2014; Rundlöf et al. 2015; Woodcock et al. 2017), nor all developmental stages of bees (Atkins & Kellum 1986), are equally sensitive to all pesticides. Neonicotinoids are often more toxic to bees when exposure is via ingestion (Suchail et al. 2001; Iwasa et al. 2004; Godfray et al. 2014, 2015), and solitary bees are more sensitive than either honey bees or bumble bees to oral exposure (Devillers et al. 2003; Rundlöf
et al. 2015; Woodcock et al. 2017), therefore the use of oral honey bee LD\textsubscript{50} values in this study may under-represent oral toxicity to hoary squash bees. However, toxicity via contact exposure varies much less among species (Decourtye & Devillers 2012; Arena & Sgolastra 2014), thus honey bee contact LC\textsubscript{50} values may adequately represent contact toxicity for adult female hoary squash bees, especially because the two species have similar body size. The evaluation of hazard to larval squash bees using adult honey bee LD\textsubscript{50} or LC\textsubscript{50} values in this study may be an underestimation of hazard because of species and developmental stage differences in sensitivity to neonicotinoids (Godfray et al. 2014, 2015; Bonmatin et al. 2015).

Pesticides

Although the hazard from thiamethoxam appears to be relatively high (HQ = 9.41), this is strongly influenced by a single detection in soil in which relatively high residues (6.8 ng a.i./g soil) were found. Although only one sample had detectable concentrations of thiamethoxam, clothianidin residues detected in soil samples could in fact be the breakdown products of applied thiamethoxam, rather than of applied clothianidin itself (Bonmatin et al. 2015; Hilton et al. 2016).

Our results suggest that both clothianidin (HQ = 1.93) and imidacloprid (HQ = 3.15) pose a hazard in Cucurbita growing systems in Ontario because they are both detected frequently, and their respective HQs exceed 1 when summed across all exposure matrices. Imidacloprid appears to be substantially more hazardous in this cropping system than clothianidin based on this deterministic approach. The combined hazard of all neonicotinoids in the system was also high (HQ\textsubscript{combined} = 14.49 including thiamethoxam; HQ\textsubscript{combined} = 5.08 without thiamethoxam), meaning that hoary squash bee populations may be at hazard of exposure to lethal concentrations of neonicotinoids in a worst-case scenario. As such, further risk analysis using a probabilistic approach of the neonicotinoids thiamethoxam, clothianidin, and imidacloprid was warranted.

Although combined hazard from chlorantraniliprole in all matrices was low (HQ = 0.11) it was included in further probabilistic risk analysis for comparative purposes because it is a persistent, systemic insecticide that was detected frequently (24% soil samples) in Cucurbita growing systems in Ontario.
Exposure Matrices
There is common agreement that bees can be exposed to neonicotinoids from nectar and pollen in the flowers upon which they forage (EFSA 2012; Godfray et al. 2014, 2015). However, there are currently no studies that evaluate the risks to ground-nesting bees from direct exposure to neonicotinoids in soil, although some studies evaluating direct effects on other soil fauna exist (de Lima e Silva et al. 2017). This study demonstrates that there is low hazard to adult hoary squash bees from pesticide exposure via nectar (HQ = 0.18), or to adult and larval hoary squash bees from pollen (HQ_{adult + larva} = 0.36), during their period of foraging and nesting activity in Ontario despite their specialized diet. In comparison, hazard quotients associated with pesticide residues from the pollen loads collected by honey bees, an example of a diet generalist, ranged from 0.01 to 75,000 (Stoner & Eitzer 2013). Such wide differences in HQs likely reflect the significant variability in the pesticide residues found in nectar and pollen of different crops, and even among varieties of the same crop (Bonmatin et al. 2015).

Because both imidacloprid and thiamethoxam (and the resulting breakdown metabolite, clothianidin) can be applied directly to the soil in Cucurbita cropping systems, and may persist in the soil for longer than a single growing season in Canada (MOECC 2016), it is unsurprising that the hazard to the ground-nesting hoary squash bee from neonicotinoids in soil (HQ_{soil} = 12.99) is much higher than even the combined hazard from both pollen and nectar (HQ_{pollen+nectar} = 0.54) in this cropping system. Even if we remove the contribution of thiamethoxam, whose hazard may be over-inflated in this study, the hazard from soil remains high (HQ_{soil, thiamethoxam removed} = 3.58) and appears to be the most important route of exposure for hoary squash bees in Ontario.

Developmental Stage
Our analysis of hazard by developmental stage in this study excluded the hazard to adult male hoary squash bees from pollen and nectar consumption and hazard to larval stages from consumption of nectar and contact with their pollen provisions. Both adult male hoary squash bees and larvae can be expected to consume much less nectar than adult females because they are not involved in energy-expensive foraging activities to provision nest cells, nor are they involved in nest excavation and construction. For honey bees, larvae are reported to consume 150% less nectar than adult pollen foragers (Rortais et al. 2005). Furthermore, male hoary squash bees spend about 18 hours per day sleeping in wilted Cucurbita blossoms (Willis and Kevan 1995),
whereas females are active throughout the daylight hours, engaged in foraging activities in the morning and nest excavation in the afternoon (Hurd & Linsley 1974).

Hazard to larval hoary squash bees was not particularly high from their main exposure matrix, consumption of pollen (HQ_{larvae, pollen} = 0.06), and would not likely be high from nectar because relative consumption by adult females is much higher than it would be for larvae (Rortais et al. 2005) and HQ_{nectar, adult female} was less than 1. This study assumes that larval hoary squash bees are protected from direct exposure to neonicotinoids in soil (both adhering to soil particles and in soil water) because of the waterproof nature of the lining of hoary squash bee nest cells (Mathewson 1968), an assumption that requires further investigation.

The combined hazard for adult female hoary squash bees from all exposure matrices (soil, pollen, nectar) is high (HQ_{adult female} = 5.03 excluding thiamethoxam), of which 91% is attributable to exposure to neonicotinoids in soil (HQ_{adult female, soil} = 4.58; 4.58/5.03 = 91%). Thus, adult female hoary squash bees are at hazard of being exposed to lethal doses of clothianidin and/or imidacloprid as they complete construction of a single nest. Mathewson (1968) suggests that hoary squash bees can construct more than one nest per season when environmental conditions (e.g. nectar and pollen resources, weather) permit. However, in this study, female hoary squash bees were already exposed to doses above lethal levels of both imidacloprid and clothianidin (HQs >1) as they constructed a single nest, rendering it unlikely they would be able to construct a second. Indeed, under present soil neonicotinoid residue conditions in Ontario Cucurbita cropping systems, pesticide exposure conditions may preclude the construction of more than one nest in a season even if all other conditions are favourable.

In summary, hazard appears to be low for larval hoary squash bees in Cucurbita cropping systems in Ontario, though this conclusion depends upon the assumed protection provided to them by their waterproof nest cell lining during development. For adult female hoary squash bees, the hazard was relatively high and mostly attributable to neonicotinoid residues in soil.

**Probabilistic Risk Analysis**

Hoary Squash Bee in Cucurbita System

For the hoary squash bee, it is clear from the EEDs for acute exposure (48 h, 2.23 g soil; Figure 3, Table S2), that probability of exceedance of the mean honey bee LC\textsubscript{50} is below the acceptable
5% threshold for imidacloprid, clothianidin and chlorantraniliprole. If the solitary bee surrogate LC50 endpoint is used, both imidacloprid (31.2%) and clothianidin (28.3%) residues in soil pose an unacceptable risk to the hoary squash bee (Figure 3, Table S2).

The chronic exposure scenario presented a more troubling risk picture because the amount of exposure to soil was much greater (30 days, 33.5 g soil) than in the acute exposure scenario (Figure 4). In the chronic exposure scenario, imidacloprid and clothianidin pose an unacceptably high risk of exceedance for all exposure endpoints (mean honey bee LC50, least honey bee LC50, and solitary bee surrogate LC50: Figure 4A,B), whereas risk from chlorantraniliprole exceeds the 5% threshold for only the solitary bee surrogate LC50 (Figure 4C). It is very probable that at least some of the clothianidin found in Cucurbita crops systems may in fact be metabolites from the application of thiamethoxam to soil (Bonmatin et al. 2015). For example, ~3-46 % of the residues recovered from soil sampled more than 60 days after thiamethoxam application have been found to be the metabolite, clothianidin (Hilton et al. 2016). Further work on the fate of thiamethoxam in Cucurbita crop fields is required to determine whether seed-applied or soil-applied thiamethoxam poses a risk to hoary squash bees via its metabolite, clothianidin.

There were at least three issues generating uncertainty when attempting to assess the potential risk posed by neonicotinoid residues in soil to hoary squash bees. Firstly, the lack of information about insecticide toxicity for this species, or indeed any other solitary ground-nesting surrogate species. As the hoary squash bee is similar in size to the honey bee it may be well represented by the available toxicity data for honey bees, especially for contact exposure which tends to vary less among species than measures of oral exposure (Decourtye & Devillers 2012). However, this has not yet been empirically established for the hoary squash bee. Secondly, although soil-applied neonicotinoids are known to elicit negative effects on Lepidoptera that pupate in soil (Dilling et al. 2009), Carabid beetles that live in soil at all life stages (Kenkel et al. 2001) and Hexapoda, Collembola, Thysanoptera, and Coleoptera adults (Peck 2009), we could find no information on the extent to which insecticide residues in soil can pass through the cuticle or into spiracles of insects that burrow in soil. In this study we assumed a worst-case scenario in which all the soil residues are translocated during exposure, but this is unlikely, even though neonicotinoids have relatively low organic carbon-water partition coefficients (Koc; Table 5; Wettstein et al. 2016). Nevertheless, if even if 50% of the neonicotinoids from the chronic exposure scenario were bioavailable, exceedance of the solitary bee surrogate LC50 would still be
substantially higher than the 5% threshold for both clothianidin (38.8%) and imidacloprid (33.2%). Lasty, the probabilistic risk assessment has certain limitations. Despite low detection limits for soil reported by the Guelph University Agri-Food Labs, there is a need for even lower detection limits in soil to bring them in line with exposure concentrations corresponding to the lowest honey bee LC$_{50}$, the NOAEC, or the solitary bee surrogate LC$_{50}$ (Table S1) for some compounds. Confidence intervals for the environmental exposure distributions in this study are comparatively wide due to limited sample size (n = 29) and high limits of detection, and results must be understood within this context. However, this study is valuable and significant because it represents the first attempt to evaluate risk from insecticide residues in soil for any ground-nesting bee. Interestingly, even at the extreme high values for confidence intervals (where exceedance would be lowest), exceedance remains above the acceptable limit (5%) for the solitary bee surrogate lethal dose endpoint for clothianidin and imidacloprid in both the acute and chronic scenarios (Figures 3, 4).

All Ground-Nesting Bees in Field Crop System
About 70% of solitary bees in eastern Canada nest in the ground (Packer et al. 2007), many of which (including species in the genera Agapostemon, Andrena, Anthophora, Colletes, Eucera, Halictus, Lasioglossum, Megachile and Melissodes) are associated with agriculture (Pindar et al. 2017; USDA 2017). Because there is little information about exposure to soil for most ground-nesting bees, this study used exposure of the hoary squash bee to the soil matrix during nest construction as a surrogate for exposure in all ground-nesting bees as a first step. It is interesting to compare our estimates of soil exposure of 33.5g for a hoary squash bee female excavating a single nest (using nest architecture data from Mathewson 1968) with other ground-nesting bee species for which nest data exist. For example, total exposure to soil for Andrena prunorum females is estimated to be 30.23 g using nest dimensions described by Milczky (2008), and the amount of soil excavated by the female alkali bee (Nomia melanderi) when building nests is estimated to be 26.3 ± 5.7 g (Cane 2003). 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, and highlights that there is potential for appreciable variability in exposure via soil across species. Using the amount of soil exposure for the hoary squash bee as a surrogate for all ground-nesting bee species may therefore have its limitations.
Ground-nesting bees vary greatly in size (Michener 2007), and many are much smaller than hoary squash bees or honey bees. These smaller bees may have lower exposure to neonicotinoids in soil because they construct narrower tunnels and nest cells to fit their smaller bodies (Michener 2007), therefore contact smaller soil volumes overall. This could be counteracted by interspecific differences in sensitivity to residues to which they are exposed based on body size and other factors (Devillers *et al.* 2003;Arena & Sgolastra 2014; Sgolastra *et al.* 2018). However, until more information emerges, using the hoary squash bee as a surrogate for all ground-nesting bees is the best model available to evaluate risk from exposure to neonicotinoid residues in agricultural soil.

Neonicotinoid residues detected in Ontario’s agricultural soils reflect variation in usage for different crops. For *Cucurbita* crops, imidacloprid and clothianidin are the most commonly detected neonicotinoids with very little detection of thiamethoxam. For agricultural soils where field crops such as corn and soybeans are grown, residues of clothianidin and thiamethoxam are more commonly detected, with fewer imidacloprid residues. Clothianidin was found in 96.34% of agricultural soil samples taken from field cropping areas in the spring following the year of application, suggesting that exposure to clothianidin residues for bees nesting in agricultural soils is likely to be both ubiquitous and chronic. This is not surprising as clothianidin-treated seeds are commonly used in the production of field corn in Ontario with about 1.5 million acres planted with clothianidin-treated seed in the 2015-2016 season (MOECC 2017). The resulting EED for clothianidin in Ontario’s agricultural soils in the spring, before a new cropping cycle, shows unacceptable likelihoods of exceedance of all the exposure endpoints for both the acute (Figure 5) and chronic (Figure 6) exposure scenarios for ground-nesting bees. For the acute scenario, the lowest exceedance (honey bee LC$_{50}$ exposure endpoint) is 11.72%, more than twice the acceptable threshold level, and the exceedance for the solitary bee surrogate LC$_{50}$ endpoint is extremely high (81.85 %: Figure 5). Exceedances for the chronic exposure scenario are greater than those for the acute scenario. As clothianidin-treated seeds are planted in a new cropping cycle, these exceedances can be expected to increase as neonicotinoids are released into the soil.

Ground-nesting solitary bees from 13 genera have been collected in corn and soybean fields in Iowa (Wheelock & O’Neal 2016; Wheelock *et al.* 2016). Although this does not conclusively prove that these species were nesting within fields, the small foraging ranges of solitary bees (Gathmann & Tscharntke 2002) would suggest that many likely were. Different bees are active
at different times during the season (Packer et al. 2007; Richards et al. 2011). Those species active in the early spring may only be exposed to the minimum residue concentrations described here, but those that are active in the summer, or throughout the season, may be exposed to much higher neonicotinoid concentrations in soil.

Risk to ground-nesting bees from exposure to clothianidin residues in the field crop soil is high, and action should be taken to mitigate that risk to preserve the pollination services of these bees. When evaluating risk to ground-nesting bees from soil-applied neonicotinoids, it is important to differentiate between clothianidin residues which originate from direct applications of clothianidin and those that originate from the breakdown of thiamethoxam applications. As thiamethoxam is broken down into clothianidin relatively quickly in soil (Bonmatin et al. 2015), one mitigation strategy would be to reduce use of thiamethoxam-treated seed.

Risk to ground-nesting bees from acute exposure to thiamethoxam residues in field crop soil in Ontario is below the acceptable level of exceedance (i.e. 5%) for all exposure endpoints, except the solitary bee surrogate LC50 (25.56 %: Figure 5). However, for the chronic exposure scenario, risk to ground-nesting bees from thiamethoxam residues in field crop soil is high for all endpoints (Figure 6). Thiamethoxam is used as a seed treatment on both field corn and soybean crops in Ontario and was applied to 1.98 million acres in the 2015-2016 season (MOECC 2017). The apparently lower risk to ground-nesting bees associated with thiamethoxam may be the product of its shorter environmental half-life (Table 5) or, as stated earlier, its tendency to break down quickly into clothianidin in soil (Bonmatin et al. 2015).

Risk to ground-nesting bees from acute exposure to imidacloprid residues in field crop soil in Ontario is below the acceptable level of exceedance (i.e. 5%) for all exposure endpoints (Figure S1). For the chronic exposure scenario, exceedance is greater than 5% for only the solitary bee surrogate LC50 (9.24 %: Figure S2). The lower risk to ground-nesting bees associated with imidacloprid in soil may be because use of this insecticide as a seed treatment for these field crops is much lower (317,253 acres, 7% of total seed-treated field crop acreage). This differs substantially from the situation in Cucurbita-crops, which rely heavily on soil-applied imidacloprid and show greatest risk to hoary squash bees from imidacloprid residues. This indicates that one of the main concerns around neonicotinoid insecticide exposure for ground-nesting bees is their use in soil applications as either treated seed or in-furrow soil drenches. This
use has been partially mitigated in Ontario by increased regulation of the sale of neonicotinoid-treated corn and soybean seed (OMAFRA 2015) but has not been addressed for other crops.

**Conclusions**

Neonicotinoid residues in soil may pose a considerable risk to female hoary squash bees as they construct their nests in *Cucurbita*-crop growing systems. Furthermore, if squash bees are used as a surrogate for all ground-nesting species that are found in association with agriculture, then all these species should be considered at risk of harm from exposure to neonicotinoids in soils of neonicotinoid-treated field crops (e.g. corn and soy) in Ontario, based on pesticide residue levels detected in the MOECC data set. There are still areas of uncertainty, and further work is needed to determine the sensitivity of the hoary squash bee to neonicotinoids relative to the honey bee and to explicitly determine larval hoary squash bee exposure in soil. Advances in analytical techniques are also needed to allow for detection of lower residue concentrations in soil and to bring detection more in line with exposure endpoints for ground-nesting solitary bees. Recognition and mitigation of risks to ground-nesting bees from exposure to neonicotinoid residues in agricultural soil are needed to protect these important crop pollinators and inform pesticide use guidelines.

**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, 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 Excellent 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.

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Figure 1. Nest of a hoary squash bee (Peponapis pruinosa) showing an adult female excavating a lateral tunnel and 4 immature stages (larvae) in sealed nest cells. Each nest cell is coated with a water-resistant lining. Soil from the main tunnel is moved to the soil surface and soil from lateral tunnels is backfilled into the vertical tunnel. The length of lateral tunnels varies. Graphic produced
by Ann Sanderson and reproduced with permission.
Figure 2. Percentage of samples with detectable insecticides and fungicides in three exposure matrices (pollen, nectar and soil) from 18 farms growing Cucurbita crops in Ontario, Canada in 2016 (n = 29 samples). Limits of detection and quantification (LOD/LOQ) for each compound are listed in Table 3.
Figure 3. Environmental Exposure Distribution (EED) for (A) clothianidin and (B) imidacloprid concentrations measured in soil samples taken from 0-15 cm depth in Cucurbita-crop fields in Ontario, 2016. Effects benchmark concentrations for acute exposure (48 h, 2.23 g soil) for the hoary squash bee, *Peponapis pruinosa* (i.e. honey bee geometric mean LC$_{50}$, honey bee lowest LC$_{50}$, honey bee NOAEC, solitary bee surrogate LC$_{50}$), are represented by vertical lines on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from 1.0. Grey horizontal lines represent individual samples below the limit of detection.
Figure 4. Environmental Exposure Distribution (EED) for (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 LC$_{50}$, honey bee NOAEC, solitary bee surrogate LC$_{50}$), are represented by vertical lines on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from 1.0. Grey horizontal lines represent individual samples below the limit of detection.
Figure 5. Environmental Exposure Distribution (EED) for (A) clothianidin and (B) thiamethoxam in soil from field crops (corn, soybeans, wheat) based on MOECC data. Soil samples taken from 0-15 cm depth from agricultural soil in southern Ontario, 2016. Effects benchmark concentrations for acute exposure (48 h, 2.23 g soil) for solitary ground-nesting bees are based on hoary squash bee surrogate exposure amounts. Effects measures (i.e. honey bee geometric mean LC50, honey bee NOAEC, solitary bee surrogate LC50) are represented by vertical lines on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from 1.0. Grey horizontal lines represent individual samples below the limit of detection.
Figure 6. Environmental Exposure Distribution (EED) for (A) clothianidin and (B) thiamethoxam in soil from field crops (corn, soybeans, wheat) based on MOECC data. Soil samples taken from 0–15 cm depth from agricultural soil in southern Ontario, 2016. Effects benchmark concentrations for chronic exposure (30 days, 33.5 g soil) for solitary ground-nesting bees are based on hoary squash bee surrogate exposure amounts. Effects measures (i.e. honey bee geometric mean LC$_{50}$, honey bee NOAEC, solitary bee surrogate LC$_{50}$) are represented by vertical lines on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from 1.0. Grey horizontal lines represent individual samples below the limit of detection.
Figure S1. Environmental Exposure Distribution (EED) for acute exposure (48h, 2.23 g soil) to imidacloprid in soil for solitary ground-nesting bees. Based on MOECC data and hoary squash bee surrogate exposure amounts. Soil samples taken from 0-15 cm depth from agricultural soil in southern Ontario, 2016. Effects benchmark concentrations for acute exposure (48 h, 2.23 g soil) for the hoary squash bee, *Peponapis pruinosa* (i.e. honey bee geometric mean LC$_{50}$, honey bee NOAEC, solitary bee surrogate LC$_{50}$) are represented by vertical lines on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from 1.0. Grey horizontal lines represent individual samples below the limit of detection.
Figure S2. Environmental Exposure Distribution (EED) for chronic exposure (30 days, 33.5 g soil) to imidacloprid in soil for solitary ground-nesting bees. Based on MOECC data and hoary squash bee surrogate exposure amounts. Soil samples taken from 0-15 cm depth from agricultural soil in southern 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 LC$_{50}$, honey bee NOAEC, solitary bee surrogate LC$_{50}$) are represented by vertical lines on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from 1.0. Grey horizontal lines represent individual samples below the limit of detection.
Table 1. Calculations of the volume, and therefore, mass of soil excavated by a female hoary squash bee (*Peponapis pruinosa*) to construct an underground nest, from which to determine potential insecticide exposure via soil. Nest dimensions follow data from Mathewson (1968).

| Part of Nest                  | Soil volume/ mass |
|------------------------------|-------------------|
| a. Main vertical shaft       | 6.92 cm³          |
| (7 mm diameter x 18 cm long) |                   |
| b. Antechamber               | 2.31 cm³          |
| (7 mm diameter x 6 cm long)  |                   |
| c. 5 brood cells             | 14.04 cm³         |
| (7 mm diameter x 7.3 cm long = 2.81 cm³ each) | |
| d. 5 brood caps              | 1.92 cm³          |
| (7 mm diameter x 1 cm thick = 0.3847 cm³ each) | Estimated from our own observations |
| Total volume of soil excavated (a + b + c + d) | 25.19 cm³ |
| Total mass of soil excavated (Total volume x Bulk density (BD) of loam; BD = 1.33 g/cm³) | **33.51 g** |
Table 2. Summary of pesticide exposure routes, exposure types, exposure amount, and exposure period for the hoary squash bee (*Peponapis pruinosa*).

| Exposure Route | Developmental Stage | Exposure type | Exposure amount | Period of Exposure |
|----------------|---------------------|---------------|-----------------|--------------------|
| Soil           | Adult Female        | Contact       | 33.51 g<sup>a</sup> | Construction of 1 nest (~30 days) |
| Soil           | Larva               | Contact       | Putatively not exposed<sup>a</sup> | 10 months |
| Nectar         | Adult Female        | Oral          | <<780 mg; based on pollen-foraging honey bee<sup>b</sup> | 30+ days |
| Nectar         | Adult Male          | Oral          | Unknown, <adult female | 30+ days |
| Nectar         | Larva               | Oral          | Unknown, < adult females | 15 days |
| Pollen         | Larva               | Oral          | 49.2 mg<sup>c</sup> | 15 days |
| Pollen         | Larva               | Contact       | Unknown          | 15 days |
| Pollen         | Adult Female        | Contact       | 246 mg (5x larval exposure) | 30 days |
| Pollen         | Adult Female        | Oral          | Unknown          | During oocyte maturation<sup>d</sup> |

<sup>a</sup> Mathewson 1968  
<sup>b</sup> Rortais *et al.* 2008  
<sup>c</sup> Willis 1991 & present study  
<sup>d</sup> Michener 2007
Table 3. All residues detected in soil, pollen, and nectar of *Cucurbita*-crop growing systems in Ontario, Canada, 2016, showing limits of detection and quantification (LOD/LOQ), maximum concentrations, geometric mean concentrations, and frequency of detection for each residue (n = 29 samples, nd = not detected). Frequency of detection is based on samples in which residues were detectable but not necessarily quantifiable (>LOD).

| Type of pesticide | Active ingredient | Soil LOD/LOQ | Soil Max Conc. (ppb) | Soil Mean Conc. (ng a.i./g matrix) | Soil Freq. Detection | Pollen LOD/LOQ | Pollen Max Conc. (ppb) | Pollen Mean Conc. (ng a.i./g matrix) | Pollen Freq. Detection | Nectar LOD/LOQ | Nectar Max Conc. (ppb) | Nectar Mean Conc. (ng a.i./g matrix) | Nectar Freq. Detection |
|-------------------|-------------------|--------------|----------------------|-----------------------------------|----------------------|-----------------|------------------------|-------------------------------|----------------------|---------------|-----------------------|----------------------------------------|---------------------|
| Insecticide       | Clothanidin       | 1/4          | 5.9                  | 2.0                              | 34                   | 2/8             | nd                     | nd                           | 0                    | 1/4           | nd                     | nd                       | 0                   |
|                   | Imidacloprid      | 3/9          | 41.6                 | 3.0                              | 21                   | 2/8             | 4.3                    | 4.3                          | 3                    | 1/4           | 1.1                    | 0.9                      | 3                   |
|                   | Thiamethoxam      | 5/20         | 6.8                  | 6.8                              | 3                    | 2/8             | nd                     | nd                           | 0.6/2                | nd             | nd                     | nd                       | 0                   |
|                   | Chlorantraniliprole | 6/20       | 148.5                | 36.8                             | 24                   | 3/10            | 68                     | 68                           | 3                    | 2/6           | nd                     | nd                       | 0                   |
|                   | Carbaryl          | 1/4          | 352.8                | 14.2                             | 10                   | 2/7             | 31.1                   | 16.5                         | 7                    | 0.6/2         | nd                     | nd                       | 0                   |
|                   | Methomyl          | 7/20         | nd                   | nd                               | 0                    | 2/6             | nd                     | nd                           | 0.3/0.9              | 0.5            | 0.4                    | 7                        | 7                   |
|                   | Dimethoate        | 2/6          | nd                   | nd                               | 0                    | 0.5/2            | nd                     | 6.2                           | 3                    | 0.4/1         | 0.5                    | 0.5                      | 3                   |
| Fungicide         | Pyraclostrobin    | 4/10         | 16.7                 | 3.8                              | 10                   | 1/4             | 399.6                  | 29.6                          | 7                    | 0.4/1         | 2                      | 2                        | 3                   |
|                   | Picoxystrobin     | 0.4/1        | nd                   | nd                               | 0                    | 0.7/2            | 110.2                  | 4.6                           | 10                   | 0.2/0.7       | 0.3                    | 0.3                      | 3                   |
|                   | Boscalid          | 3/9          | 374.9                | 46.2                             | 31                   | 2/6             | 25                     | 17.8                          | 7                    | 2/8           | nd                     | nd                       | 0                   |
|                   | Propamocarb       | 20/50        | 64.4                 | 23.0                             | 10                   | 0.9/3           | 402.6                  | 222.1                         | 14                   | 0.2/0.5       | 74.5                   | 11.2                     | 17                  |
|                   | Quinoxyfen        | 5/10         | 14.7                 | 7.9                              | 3                    | 3/8             | 94.9                   | 79.1                          | 7                    | 1/3           | nd                     | nd                       | 0                   |
|                   | Difenconazole     | 4/10         | 40.7                 | 18.9                             | 14                   | 2/5             | 22.4                   | 16.5                          | 7                    | 0.7/2         | nd                     | nd                       | 0                   |
| Herbicide         | Napropamide       | 1/4          | 59.4                 | 2.8                              | 7                    | 0.4/1           | 5.0                    | 5.0                           | 3                    | 0.1/0.4       | nd                     | nd                       | 0                   |
|                   | Linuron           | 5/10         | 0.8                  | 0.8                              | 0                    | 1/4             | nd                     | nd                           | 0                    | 0.8/2         | nd                     | nd                       | 0                   |
Table 4. 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 LD\textsubscript{50} values (taken from US-EPA database, unless otherwise noted), 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 in samples. a.i. = active ingredient.

| LC\textsubscript{50} Endpoint (Honey bee) | SOIL | POLLEN | NECTAR | COMBINED HAZARD QUOTIENT |
|---------------------------------|------|--------|--------|--------------------------|
| Contact Oral Mean Conc. in Matrix | HQ Adult Female Mean Conc. in Matrix | HQ Larvae Mean Conc. in Matrix | HQ Adult Female Mean Conc. in Matrix | Across Exposure Matrix Adult Female Larvae |
| ng a.i./bee ng a.i./bee ng a.i./g | =33.5 g/bee *Matrix Conc. /LD\textsubscript{50} | =0.0492 g/bee *Matrix Conc. /LD\textsubscript{50} | =5*0.0492 g/bee* Matrix Conc. /LD\textsubscript{50} | ng a.i./g | =0.78 g/bee* Matrix Conc. /LD\textsubscript{50} | ∑HQ | ∑HQ | ∑HQ |
| INSECTICIDE ∑HQ |
| Clothianidin (geomean) \textsuperscript{1} | 35.88 | - | 1.95 | 1.93 | nd | 0 | 0 | nd | 0 | 0 | 1.93 |
| Imidacloprid (geomean) \textsuperscript{2} | 40.03 | 3.90 | 2.99 | 2.65 | 4.3 | 0.05 | 0.27 | 0.88 | 0.18 | 15.11 | 15.05 | 0.06 |
| Thiamethoxam (geomean) \textsuperscript{3} | 25.64 | - | 6.8 | 9.41 | nd | 0 | 0 | nd | 0 | 9.41 |
| Chlorantraniliprole \textsuperscript{4} | 12500 | - | 36.82 | 0.10 | 68 | 2.68E-4 | 1.34E-3 | nd | 0 | 0.11 |
| Carbaryl \textsuperscript{5} | 1100 | - | 14.2 | 0.46 | 16.47 | 7.37E-4 | 3.68E-3 | nd | 0 | 0.46 |
| Dimethoate \textsuperscript{5} | - | 56.00 | nd | 0.00 | 6.20 | 5.45E-3 | 2.72E-2 | 0.50 | 0.01 | 0.04 |
| Methomyl \textsuperscript{5} | - | 290.00 | nd | 0.00 | 6.20 | 5.45E-3 | 2.72E-2 | 0.50 | 0.01 | 0.04 |
| FUNGICIDE ∑HQ |
| Pyraclostrobin \textsuperscript{3} | 100000 | - | 3.8 | 0.00 | 29.65 | 1.46E-5 | 7.29E-5 | 2 | 1.56E-5 | 0.00 |
| Picloxystrobin \textsuperscript{5} | 200000 | - | nd | 0.00 | 4.55 | 1.12E-6 | 5.60E-6 | 0.3 | 1.17E-06 | 0.00 |
| Boscalid \textsuperscript{5} | 200000 | 166000 | 46.22 | 0.01 | 17.82 | 5.28E-6 | 2.64E-5 | nd | 0 | 0.01 |
| Propamocarb \textsuperscript{5} | 100000 | 116000 | 23.03 | 0.01 | 222.06 | 9.42E-5 | 4.71E-4 | 11.18 | 7.52E-05 | 0.01 |
| Quinoxyfen \textsuperscript{5} | 100000 | 100000 | 7.86 | 0.00 | 79.14 | 3.89E-6 | 1.95E-5 | nd | 0 | 0.00 |
| Difenconazole \textsuperscript{5} | 100000 | 177000 | 18.87 | 0.01 | 16.46 | 4.58E-6 | 2.29E-5 | nd | 0 | 0.01 |
| Napropamide \textsuperscript{6} | - | 113500 | 3.6 | 0.00 | 0.3 | 1.30E-7 | 6.30E-7 | nd | 0 | 0.00 |

\textsuperscript{1}US-EPA database; Iwasa et al. 2004; EC database; Sanchez-Bayo & Goka 2014
\textsuperscript{2}US-EPA database; Iwasa et al. 2004; Sanchez-Bayo & Goka 2014; Rhuzhong et al. 1991; Stark et al. 1995
\textsuperscript{3}US-EPA database; EC database; Sanchez-Bayo & Goka 2014; Goulson et al. 2008
\textsuperscript{4}Dinter et al. 2009
\textsuperscript{5}US-EPA database
\textsuperscript{6}Stoner & Eitzer 2013
Table 5. Physical, chemical, and environmental fate properties of imidacloprid, clothianidin, thiamethoxam and chlorantraniliprole

| Active Ingredient | Imidacloprid | Clothianidin | Thiamethoxam | Chlorantraniliprole |
|-------------------|--------------|--------------|--------------|---------------------|
| Chemical Name a   | 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine | (E)-1-(2-chloro-1, 3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine | 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-N-nitro-4H-1,3,5-oxadiazin-4-imine | 3-bromo-N-(4-chloro-2-methyl-6-((methylamino)carbonyl)phenyl)-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide |
| Insecticide class | Neonicotinoid | Neonicotinoid | Neonicotinoid | Anthranilic diamide |
| Formula a          | C₉H₁₀ClN₅O₂  | C₆N₅H₈SO₂Cl  | C₈H₁₀ClN₅O₃S | C₁₈H₁₄BrCl₂N₅O₂   |
| CAS No. a          | 138261-41-3 | 210880-92-5  | 153719-23-4  | 500008-45-7        |
| Molecular Weight l (g/mol) a | 255.69 | 327 | 297.1 | 483.2 |
| Solubility in water at 20°C, pH 7 (mg/L) a | 610 a | 327 b | 4100 a | 0.9-1.0 a |
| Adsorption to Particles (soil) Koc at pH 7 a | 156-800 | 60 | 68.4 | 244-468 |
| Volatility (air) Vapour pressure a (mm Hg at 25°C) | 7.0x10⁻¹² | 9.8x10⁻¹⁰ | 4.95x10⁻¹¹ | 1.2x10⁻¹⁴ |
| Log Kow a          | 0.57 | 0.7 | -0.13 | 2.76 |
| Bio-degradation DT50 (days) | 48-190 a | 148-1155 a | 46.3-301 c | 60-365 |

a TOXNET Toxicology Data Network. https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm. Accessed June 12, 2018.
b USEPA/Office of Pesticide Programs; New Pesticide Fact Sheet—Clothianidin (May 2003). http://www.epa.gov/opprd001/factsheets/ Accessed July 24, 2018
c Gupta et al., 2008
Table S1. Exposure endpoints for amounts of soil handled by the hoary squash bee in acute (48h, 2.23 g soil) and chronic (30 days, 33.5 g soil) exposure scenarios based on various effect endpoints in the scientific literature (a. US-EPA database; b. Iwasa et al. 2004; c. EC database; d. Sanchez-Bayo & Goka 2014; e. Arena & Sgolastra 2014; f. Rhuzhong et al. 1991; g. Stark et al. 1995; h. Goulson et al. 2008; i. Dinter et al. 2009). HB = honey bee, SB = solitary bee.

| Insecticide       | Exposure type | Effect endpoint | Effect endpoint concentration (ng a.i./bee) | Exposure amount (g soil/bee) | Exposure endpoint (A/B) (ng a.i/g soil) | Source for effect endpoint |
|-------------------|---------------|-----------------|---------------------------------------------|------------------------------|----------------------------------------|---------------------------|
| Clothianidin      | Acute         | Geomean HB LD₅₀ | 35.88                                      | 2.23                         | 15.16                                  | a-d                       |
|                   |               | Lowest HB LD₅₀  | 22                                         | 2.23                         | 9.29                                   | b                         |
|                   |               | SB Surrogate LD₅₀ | 3.588                                   | 2.23                         | 1.52                                   | e                         |
|                   |               | NOAEC           | 9.5                                        | 2.23                         | 4.01                                   |                           |
|                   | Chronic       | Geomean HB LD₅₀ | 35.88                                      | 33.5                         | 1.01                                   | a-d                       |
|                   |               | Lowest HB LD₅₀  | 22                                         | 33.5                         | 0.62                                   | b                         |
|                   |               | SB Surrogate LD₅₀ | 3.588                                   | 33.5                         | 0.10                                   | e                         |
|                   |               | NOAEC           | 9.5                                        | 33.5                         | 0.27                                   | a                         |
| Imidacloprid      | Acute         | Geomean HB LD₅₀ | 40.03                                      | 2.23                         | 16.91                                  | a,b,d,f,g                 |
|                   |               | Lowest HB LD₅₀  | 18                                         | 2.23                         | 7.61                                   | b                         |
|                   |               | SB Surrogate LD₅₀ | 4.003                                   | 2.23                         | 1.69                                   | e                         |
|                   | Chronic       | Geomean HB LD₅₀ | 40.03                                      | 33.5                         | 1.13                                   | a,b,d,f,g                 |
|                   |               | Lowest HB LD₅₀  | 18                                         | 33.5                         | 0.51                                   | b                         |
|                   |               | SB Surrogate LD₅₀ | 4.003                                   | 33.5                         | 0.113                                  | e                         |
| Thiamethoxam      | Acute         | Geomean HB LD₅₀ | 25.64                                      | 2.23                         | 10.83                                  | a,c,d,h                   |
|                   |               | Lowest HB LD₅₀  | 24                                         | 2.23                         | 10.14                                  | a                         |
|                   |               | SB Surrogate LD₅₀ | 2.564                                   | 2.23                         | 1.08                                   | e                         |
|                   | Chronic       | Geomean HB LD₅₀ | 25.64                                      | 33.5                         | 0.722                                  | a,c,d,h                   |
|                   |               | Lowest HB LD₅₀  | 24                                         | 33.5                         | 0.676                                  | a                         |
|                   |               | SB Surrogate LD₅₀ | 2.564                                   | 33.5                         | 0.072                                  | e                         |
| Chlorantraniliprole | Acute       | Lowest HB LD₅₀  | 12.50                                      | 2.23                         | 559.35                                 | i                         |
|                   |               | SB Surrogate LD₅₀ | 1250                                      | 2.23                         | 559.5                                  | e                         |
|                   | Chronic       | Lowest HB LD₅₀  | 12.50                                      | 33.5                         | 373.02                                 | i                         |
|                   |               | SB Surrogate LD₅₀ | 1250                                      | 33.5                         | 37.3                                   | e                         |
Table S2. Exceedance probabilities (i.e. the frequency that effect endpoints were exceeded), with upper and lower limits of the 95% confidence interval and the exposure concentrations associated with each effect endpoint, for all neonicotinoids detected in soil of *Cucurbita* and other agricultural field crops in Ontario for both chronic (30 days, 33.5 g soil) and acute (48h, 2.23 g soil) exposure scenarios. HB = honey bee, SB = solitary bee. Data from field crop soils taken from MOECC (2016).

| Insecticide | Exposure type | Crop system | Effect endpoint | % Exceedance | Lower limit of 95% CI | Upper limit of 95% CI | Effect concentration ng ai/g soil |
|-------------|---------------|-------------|-----------------|--------------|-----------------------|-----------------------|----------------------------------|
| Clothianidin | Chronic       | Cucurbita   | Lowest HB LC₅₀  | 44.3         | 65.9                  | 24.5                  | 0.6                              |
| Clothianidin | Acute         | Cucurbita   | Geomean LC₅₀    | 35.8         | 54.9                  | 17.7                  | 1.0                              |
| Clothianidin | Acute         | Cucurbita   | SB Surrogate LD₅₀| 68.7         | 89.1                  | 41.5                  | 0.1                              |
| Clothianidin | Acute         | Cucurbita   | HB NOAEC        | 57.1         | 79.5                  | 33.2                  | 0.3                              |
| Clothianidin | Acute         | Cucurbita   | Lowest HB LC₅₀  | 2.4          | 4.6                   | 0.4                   | 9.3                              |
| Clothianidin | Acute         | Cucurbita   | Geomean LC₅₀    | 0.5          | 1.4                   | 0.04                  | 15.2                             |
| Clothianidin | Acute         | Cucurbita   | SB Surrogate LD₅₀| 28.3         | 44.5                  | 14.2                  | 1.5                              |
| Clothianidin | Acute         | Cucurbita   | HB NOAEC        | 11.4         | 19.0                  | 4.5                   | 4.0                              |
| Imidacloprid  | Chronic       | Cucurbita   | Lowest HB LC₅₀  | 57.8         | 100                   | 26.2                  | 0.5                              |
| Imidacloprid  | Acute         | Cucurbita   | Geomean LC₅₀    | 39.8         | 99.9                  | 17.2                  | 1.1                              |
| Imidacloprid  | Acute         | Cucurbita   | SB Surrogate LD₅₀| 85.4         | 100                   | 45.4                  | 0.1                              |
| Imidacloprid  | Acute         | Cucurbita   | Lowest HB LC₅₀  | 8.9          | 17.0                  | 0.0                   | 7.6                              |
| Imidacloprid  | Acute         | Cucurbita   | Geomean LC₅₀    | 3.5          | 9.0                   | 0.0                   | 16.9                             |
| Imidacloprid  | Acute         | Cucurbita   | SB Surrogate LD₅₀| 31.2         | 95.6                  | 13.7                  | 1.7                              |
| Thiamethoxam  | Chronic       | Cucurbita   | Lowest HB LC₅₀  | 0.6          | 1.6                   | 0.0                   | 373.0                            |
| Thiamethoxam  | Acute         | Cucurbita   | Geomean LC₅₀    | 0.0          | 0.0                   | 0.0                   | NA                               |
| Thiamethoxam  | Acute         | Cucurbita   | SB Surrogate LD₅₀| 11.9         | 21.1                  | 3.69                  | 37.3                             |
| Thiamethoxam  | Acute         | Cucurbita   | Lowest HB LC₅₀  | 0.0          | 0.0                   | 0.0                   | 5595.3                           |
| Thiamethoxam  | Acute         | Cucurbita   | Geomean LC₅₀    | 0.0          | 0.0                   | 0.0                   | NA                               |
| Thiamethoxam  | Acute         | Cucurbita   | SB Surrogate LD₅₀| 0.2          | 0.7                   | 0.0                   | 559.5                            |
| Insecticide      | Exposure type | Crop system | Effect endpoint | % Exceedance | Lower limit of 95% CI | Upper limit of 95% CI | Effect concentration ng ai/g soil |
|------------------|---------------|-------------|-----------------|--------------|-----------------------|-----------------------|-------------------------------|
| Chlorantraniliprole | Chronic       | Cucurbita   | Lowest HB LC₅₀ | 0.6          | 1.6                   | 0.02                  | 373.0                        |
| Chlorantraniliprole | Chronic       | Cucurbita   | SB Surrogate LD₅₀ | 11.9         | 21.1                  | 3.7                   | 37.3                         |
| Chlorantraniliprole | Acute         | Cucurbita   | Lowest HB LC₅₀ | 0            | 0                     | 0                     | 5595.3                       |
| Chlorantraniliprole | Acute         | Cucurbita   | SB Surrogate LD₅₀ | 0.2          | 0.7                   | 0                     | 559.5                        |
| Clothianidin     | Chronic       | Field crops | Lowest HB LC₅₀ | 92.4         | 97.0                  | 86.0                  | 0.6                          |
| Clothianidin     | Chronic       | Field crops | Geomean LC₅₀   | 87.7         | 94.0                  | 80.1                  | 1.0                          |
| Clothianidin     | Chronic       | Field crops | SB Surrogate LD₅₀ | 98.8         | 99.8                  | 96.5                  | 0.1                          |
| Clothianidin     | Chronic       | Field crops | HB NOAEC       | 96.8         | 99.1                  | 92.5                  | 0.3                          |
| Clothianidin     | Acute         | Field crops | Lowest HB LC₅₀ | 27.3         | 35.3                  | 19.1                  | 9.3                          |
| Clothianidin     | Acute         | Field crops | Geomean LC₅₀   | 11.7         | 17.6                  | 5.8                   | 15.2                         |
| Clothianidin     | Acute         | Field crops | SB Surrogate LD₅₀ | 81.9         | 89.7                  | 73.6                  | 1.5                          |
| Clothianidin     | Acute         | Field crops | HB NOAEC       | 57.8         | 67.3                  | 48.7                  | 4.0                          |
| Imidacloprid     | Chronic       | Field crops | Lowest HB LC₅₀ | 5.9          | 9.7                   | 2.1                   | 0.5                          |
| Imidacloprid     | Chronic       | Field crops | Geomean LC₅₀   | 4.2          | 7.1                   | 1.3                   | 1.1                          |
| Imidacloprid     | Chronic       | Field crops | SB Surrogate LD₅₀ | 9.2          | 15.3                  | 3.9                   | 0.1                          |
| Imidacloprid     | Acute         | Field crops | Lowest HB LC₅₀ | 0.8          | 1.8                   | 0.0                   | 7.6                          |
| Imidacloprid     | Acute         | Field crops | Geomean LC₅₀   | 0.2          | 0.7                   | 0.0                   | 16.9                         |
| Imidacloprid     | Acute         | Field crops | SB Surrogate LD₅₀ | 3.3          | 5.6                   | 0.9                   | 1.7                          |
| Thiamethoxam     | Chronic       | Field crops | Lowest HB LC₅₀ | 37.4         | 45.8                  | 29.4                  | 0.7                          |
| Thiamethoxam     | Chronic       | Field crops | Geomean LC₅₀   | 35.7         | 43.95                 | 27.85                 | 0.7                          |
| Thiamethoxam     | Chronic       | Field crops | SB Surrogate LD₅₀ | 78.4         | 85.8                  | 69.7                  | 0.1                          |
| Thiamethoxam     | Acute         | Field crops | Lowest HB LC₅₀ | 0.0          | 0.3                   | 0.0                   | 10.1                         |
| Thiamethoxam     | Acute         | Field crops | Geomean LC₅₀   | 0.0          | 0.2                   | 0.0                   | 10.8                         |
| Thiamethoxam     | Acute         | Field crops | SB Surrogate LD₅₀ | 25.6         | 33.1                  | 17.8                  | 1.1                          |
Table S3. Parameters (shape and rate) and associated 95% confidence interval for the gamma model that was fit to the distribution of measured concentrations of each insecticide and the Akaike Information Criterion (AIC) for each model.

| Insecticide         | Sample depth/ cm | Crop type    | shape       | shape_2.5     | shape_97.5    | rate       | rate_2.5     | rate_97.5    | AIC         |
|---------------------|------------------|--------------|-------------|---------------|---------------|------------|---------------|---------------|-------------|
| Clothianidin        | 0-15             | Cucurbita    | 0.343044    | 0.14723308    | 0.667891188   | 0.228469   | 0.1387103    | 0.39616228   | 76.90513    |
| Imidacloprid        | 0-15             | Cucurbita    | -0.050371212| -2.517200493  | 0.910929857   | 1.589229598 | 0.216341429 | 3.155708277  | 56.83654323 |
| Thiamethoxam        | 0-15             | Cucurbita    | 1.0504021   | 1.033572828   | 1.222289678   | 0.316636   | 0.01405871   | 0.33334101   | 12.69971    |
| Chlorantraniliprole | 0-15             | Cucurbita    | 0.09636     | 0.03413813    | 0.200792325   | 0.005212   | 0.0028537    | 0.01328973   | 107.6332    |
| Clothianidin        | 0-15             | Field crops  | 1.067537    | 0.75563551    | 1.533034186   | 0.15031    | 0.0997477    | 0.23856296   | 508.4322    |
| Imidacloprid        | 0-15             | Field crops  | 0.025425    | 0.01082193    | 0.044708698   | 0.109763   | 0.0471882    | 0.39990766   | 91.718      |
| Thiamethoxam        | 0-15             | Field crops  | 0.535504    | 0.39735558    | 0.737929321   | 0.647974   | 0.4363275    | 1.09491352   | 205.267     |