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

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Using the hoary squash bee (*Peponapis pruinosa*) as a model, we provide the first probabilistic risk assessment of exposure to systemic insecticides in soil for ground-nesting bees. To assess risk in acute and chronic exposure scenarios in *Cucurbita* and field crops, concentrations of clothianidin, thiamethoxam and imidacloprid (neonicotinoids) and chlorantraniliprole (anthranilic diamide) in cropped soil were plotted to produce an environmental exposure distribution for each insecticide. The probability of exceedance of several exposure endpoints (LC_{50}s) was compared to an acceptable risk threshold (5%). In *Cucurbita* crops, under acute exposure, risk to hoary squash bees was below 5% for honey bee LC_{50}s for all residues evaluated but exceeded 5% for clothianidin and imidacloprid using a solitary bee LC_{50}. For *Cucurbita* crops in the chronic exposure scenario, exposure risks for clothianidin and imidacloprid exceeded 5% for all endpoints, and exposure risk for chlorantraniliprole was below 5% for all endpoints. In field crops, risk to ground-nesting bees was high from clothianidin in all exposure scenarios and high for thiamethoxam and imidacloprid under chronic exposure scenarios. Risk assessments for ground-nesting bees should include exposure impacts from pesticides in soil and could use the hoary squash bee as an ecotoxicology model.

Global insect pollinator declines are being driven by multiple interacting environmental stressors, including land-use intensification, pathogens, invasive species and climate change, and may threaten the production of crops that depend directly or indirectly on the pollination services that bees provide[1,2]. For bee populations living in proximity to agricultural production, exposure to pesticides is likely one of the major environmental stressors affecting population health[2,3].

Cucurbita crops (e.g., pumpkin, squash, summer squash, and gourds) are grown globally for their fruits. Because of their imperfect flowers and heavy, oily pollen, they are dependent upon bees for pollination[4]. The insecticides used to control pests in *Cucurbita* crops can also harm beneficial insect pollinators, setting up a tension between the need to control pests while maintaining healthy bee populations for the essential pollination services they provide.

In Ontario, three neonicotinoids (imidacloprid, clothianidin, and thiamethoxam) are commonly used in *Cucurbita*-crop production to control insect pests[5]. Once applied, thiamethoxam breaks down quickly to clothianidin as one of its metabolites[6,7]. Although they are effective against pests, neonicotinoid insecticides are of environmental concern because of their relatively high toxicity to (non-target) insects, their systemic nature, their persistence, and their extensive use in agriculture[8,9]. In agriculture globally, about 60% of neonicotinoids are applied as seed coatings or as in-furrow soil applications, with the remaining applied as foliar sprays[8]. Neonicotinoid residues have been found in the nectar and pollen of *Cucurbita* flowers[10,11], and in agricultural soil[3,7], where they have been found to persist across seasons[9,12,13].

For bees, both adult and larval stages may be exposed to pesticide residues consumed in nectar and pollen. The extent of exposure is likely greatest for individual adult female solitary bees because they consume pollen and nectar during sexual maturation and egg laying[14–16], consume nectar to fuel their foraging and nesting activities, and handle pollen and nectar to feed their offspring[15,17]. Male solitary bees consume nectar and pollen during

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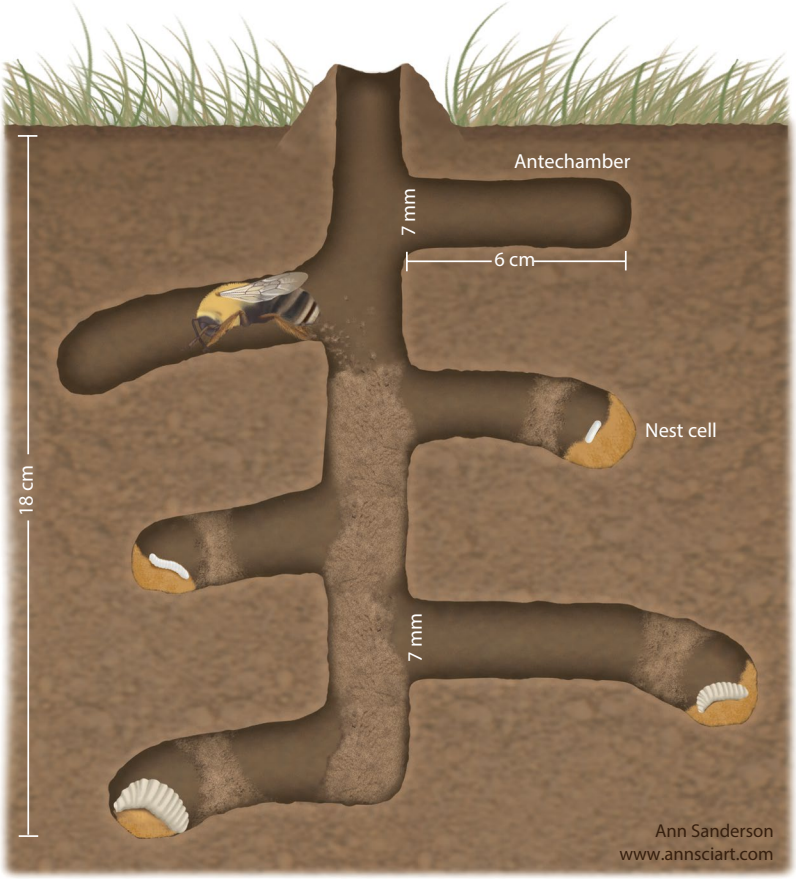

Figure 1. Nest of a hoary squash bee (*Peponapis pruinosa*) showing an adult female excavating a lateral tunnel and four immature stages (larvae) in sealed nest cells. Each nest cell is coated with a water-resistant lining. Soil from the main vertical tunnel is moved to the soil surface and soil from lateral tunnels is backfilled into the vertical tunnel. The length of lateral tunnels varies. Graphic designed by D. Susan Willis Chan, drawn by Ann Sanderson and reproduced with permission.

sexual maturation, and nectar thereafter to fuel flight[14]. Solitary bee larvae consume and topically contact pollen and nectar in their provisions[14]. For bees that do not construct their nests from glandular secretions, exposure may also be via nesting materials[16,18]. For ground-nesting bees, exposure from nesting sites is via soil contacted during nest excavation for adult females, and via contact with the soil that forms nest cells during larval development. However, larval exposure within nest cells may be precluded because of the water-resistant coating applied to nest cells by many ground-nesting species[14]. Adult male ground-nesting bees have little exposure to soil as they do not participate in nest construction[14], but they might have greater exposure to residues on plants than adult females because they may spend more time in flowers (e.g. residing inside them overnight).

The persistence of neonicotinoids in soil makes both short-term (acute) and long-term (chronic) contact exposure scenarios for ground-nesting bees plausible. Although neonicotinoid uptake from soil by bees has not yet been quantified, the translocation of neonicotinoids to bees from residues in dust generated during corn planting is well documented[19]. Although their sublethal impacts on most wild bee species are unknown, exposure to neonicotinoids can affect the transmission of information through the nervous system of both adult and larval individual bees. Such sublethal impacts on the normal physiology and behaviour of individual bees can have knock-on impacts for the function of social bee colonies and ultimately populations of both solitary and social species[20–23]. Substantial knowledge gaps remain around the toxicity and effects of neonicotinoids to arthropods, including ground-nesting bees, in soil. Furthermore, exposure routes relating to nesting materials, including soil for ground-nesting bees, are not considered as part of current regulatory risk assessments for pesticide impacts on pollinators[16].

Hoary squash bees (*Peponapis pruinosa*) are solitary bees that build their nests in the ground (Fig. 1) within *Cucurbita* cropping areas[24] and consume mostly *Cucurbita*-crop pollen and nectar[25]. In eastern North America, hoary squash bees are one of the most important pollinators of *Cucurbita* crops[26,27] and are obligately associated with these crops because they lack a wild plant host[28]. In 2014, the Pesticide Management Regulatory Agency (PMRA) of Health Canada initiated a special review of registered neonicotinoid insecticides used on cucurbit crops because of concerns about their potential impacts on hoary squash bees[29]. The hoary squash bee is common, nests in (sometimes large) aggregations, is a dietary specialist, has a well-documented natural history[17,25,26,30], and

	LD_{50} (Honey bee)		SOIL		POLLEN			NECTAR		COMBINED HAZARD QUOTIENT		
	Contact	Oral	Mean Conc. in Matrix	HQ Adult female	Mean Conc. in Matrix	HQ Larvae (Oral)	HQ Adult Female (Contact)	Mean Conc. in Matrix	HQ Adult Female	Across Exposure Matrix, All Stages	Adult Female	Larvae
	ng a.i./bee	ng a.i./bee or ppb	ng a.i./g	=33.5 g/ bee *Matrix Conc./LD_{50}	ng a.i./g	=0.0542 g/ bee * Matrix Conc./LD_{50}	=5*0.0542 g/ bee* Matrix Conc./LD_{50}	ng a.i./g	=0.78 g/ bee* Matrix Conc./LD_{50}	∑HQ	∑HQ	∑HQ
INSECTICIDE ∑HQ				**4.38**		**0.06**	**0.03**		**0.45**	**4.92**	**4.86**	**0.06**
Neonicotinoid ∑HQ				**4.32**		**0.06**	**0.03**		**0.18**	**4.59**	**4.53**	**0.06**
Clothianidin (geomean)[32,38,63,64]	35.88	—	1.95	1.82	ND	—	0	ND	0	1.82	1.82	0
Imidacloprid (geomean)[37,38,63–67]	40.03	3.9	2.99	2.50	4.3	0.06	0.03	0.88	0.18	2.76	2.71	0.06
Thiamethoxam (geomean)[32,38,63,64]	25.64	—	NQ	0	ND	0	0	ND	0	0	0	0
Chlorantraniliprole[68]	>81500	>117800	36.82	0.02	68	1.69E-5	2.26E-4	ND	0	0.02	0.02	2.26E-4
Carbaryl[63]	11200	—	14.2	0.04	16.47	—	3.99E-4	ND	0	0.04	0.04	3.99E-4
Dimethoate[63]	—	56	ND	0	6.2	0.006	—	0.5	0.007	0.01	0.007	0.006
Methomyl[63]	—	1.18	ND	0	ND	0	0	0.39	0.26	0.26	0.26	0
FUNGICIDE ∑HQ				**0.03**		**1.24E-4**	**6.18E-4**		**9.20E-5**	**0.03**	**3.08E-2**	**1.24E-4**
Pyraclostrobin[63]	100000	—	3.8	1.27E-4	29.65	—	8.04E-5	2	—	1.35E-3	1.35E-3	—
Picoxystrobin[63]	200000	—	ND	0	4.55	—	6.17E-6	0.3	—	6.17E-6	6.17E-6	—
Boscalid[63]	200000	166000	46.22	7.74E-3	17.82	5.28E-6	2.41E-5	ND	0	7.77E-3	7.77E-3	5.28E-6
Propamocarb[63]	100000	116000	23.03	7.72E-3	222.06	1.04E-3	6.02E-4	11.18	7.52E-5	9.43E-3	8.39E-3	1.04E-3
Quinoxyfen[63]	100000	1000000	7.86	2.63E-3	79.14	4.29E-5	2.14E-4	ND	0	2.85E-3	2.85E-3	4.29E-5
Difenoconazole[63]	100000	177000	18.87	6.32E-3	16.46	5.04E-5	4.46E-5	ND	0	6.37E-3	6.37E-3	5.04E-5

Table 1. Hazard quotients (HQ) for each active ingredient, and pesticide type (insecticide, fungicide) in three exposure matrices (soil, pollen, nectar) and both developmental stages (adult female or larvae) based on honey bee LD_{50} values taken from the literature[32,38,63–68] and mean residue concentration in the exposure matrices for all pesticide residues found detected on 18 *Cucurbita*-crop farms in Ontario in 2016. Numbers shown in bold face are combined hazard quotients for a pesticide type, an exposure matrix, or a developmental stage. Where "ND" is indicated, residues were not detected, where "NQ" is indicated, residues were not quantifiable in samples. a.i. = active ingredient. A dash (—) indicates no information.

is easy to maintain in captivity (D.S.W.C. personal observation), making it a promising candidate as a model species to evaluate risk of exposure to pesticides in soils for other ground-nesting solitary bee species.

This study is the first to evaluate risk of exposure to pesticides in soil for ground-nesting bees. Our aims are (1) to evaluate which pesticides and exposure matrices (soil, pollen, nectar) pose a potential hazard to hoary squash bees; (2) to determine which hoary squash bee developmental stage (adult female or larvae) is at greatest hazard; (3) to evaluate risk from agricultural soil for ground-nesting solitary bees; and (4) to contribute to risk assessments for hoary squash bees and other solitary bees.

As a first step in assessing risk, we evaluated the concentrations of all pesticides found in composite samples of soil (n = 29), nectar (n = 25), and pollen (n = 25) taken from 18 farms growing *Cucurbita* crops. For each pesticide, we related the geometric mean concentration of all samples with quantifiable concentrations to the honey bee lethal dose endpoint (LD_{50}) of that pesticide to produce a hazard quotient (HQ). Hazard quotients were summed for each route of exposure, and bee developmental stage (Table 1). Hazard quotients ≥1 flag pesticides or scenarios of concern. For flagged pesticides, and a single relevant unflagged pesticide, we undertook a probabilistic risk assessment that included all data values (including those below the limit of quantification) to determine the probability of exceeding the lethal dose. We developed Environmental Exposure Distributions (EEDs) for chronic exposure (33.5 g soil over 30 days) and acute exposure (2.23 g soil over 48 h) scenarios using honey bee (LD_{50}) and solitary bee (HB LD_{50}/10) lethal dose endpoints as benchmarks on the EEDs to calculate risk. We then applied the same approach to a publicly available data set[13] of neonicotinoid concentrations in agricultural soil to determine risk for ground-nesting bees more generally.

Results

Pesticide residue profiles-*Cucurbita* crops. Residues of 7 insecticides, 6 fungicides, and 2 herbicides were detected in samples taken from *Cucurbita* crops (Table S3). We did not assess herbicide residues further. The three exposure matrices (pollen, nectar, and soil) show different pesticide residue profiles (Table S3). In soil, 5/7 insecticides and 5/6 fungicides were detected. Pollen contained residues of 4/7 insecticides and 6/6 fungicides, but residues were detected much less frequently and at lower concentrations than in soil. Nectar contained 3/7 insecticides and 3/6 fungicides, with the lowest concentrations and frequency of detection. Imidacloprid was the only insecticide detected in all three matrices (21% of soil samples, 3% of pollen and nectar samples). Thiamethoxam

was present only in a single soil sample, at a concentration below the limit of quantification (LOQ). Clothianidin was detected in 34% of soil samples but was not detected in nectar or pollen. The insecticides chlorantraniliprole and carbaryl were detected in soil (24% and 10% of samples respectively) and pollen samples (3% and 7% respectively), but not in nectar. The insecticides methomyl and dimethoate were not detected in soil, but dimethoate was detected in pollen and nectar (3% each), and methomyl was detected in 7% of nectar samples. The fungicides pyraclostrobin and propamocarb were detected in all three matrices, whereas boscalid, quinoxyfen and difenoconazole were detected in soil and pollen only. The fungicide picoxystrobin was only detected in nectar and pollen.

Hazard assessment-hoary squash bee. *Pesticides.* Hazard quotients (HQ) for fungicide and insecticide residues in soil, pollen, and nectar for adult females and larvae based on honey bee LD_{50} values are presented in Table 1. As expected, the combined HQ for insecticides ($HQ_{insecticide\ total} = 4.92$) was much higher in all exposure matrices than for fungicides ($HQ_{fungicide} = 0.03$). Only clothianidin and imidacloprid had HQs ≥ 1 in soil, and the combined HQ of all non-neonicotinoid insecticides in soil was low ($HQ_{non\text{-}neonicotinoid} = 0.06$). Although chlorantraniliprole had an $HQ < 1$, it was included in further probabilistic risk assessment for comparison because, like neonicotinoids, it is systemic and was present in 24% of samples. When we calculated HQs based on a solitary bee surrogate LD_{50} (HB $LD_{50}/10$), the same pesticides, routes of exposure and developmental stage were flagged as hazardous, with the exception of methomyl ($HQ = 2.6$ for adult bee exposure via nectar; Table S9). The physical, chemical and environmental fate properties of the flagged neonicotinoids and chlorantraniliprole are presented in Table S4.

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

Developmental Stage. Hazard is greater for adult females ($HQ_{adult\ female} = 4.86$) than for larval hoary squash bees ($HQ_{larvae} = 0.06$), mostly because of the adult exposure to neonicotinoid residues in soil during nest construction ($HQ_{adult\ female\ soil} = 4.32$: Table 1).

Probabilistic risk assessment. *Hoary Squash Bees in Cucurbita Crops.* For the acute exposure scenario (2.23 g soil, 48 h) in soil, environmental exposure distributions (EEDs) for clothianidin showed exceedance below 5% for the geometric mean honey bee LC_{50} and the lowest honey bee LC_{50} endpoints. However, exceedance for the solitary bee surrogate LC_{50} endpoint (28.3%) was relatively high (Fig. 2a). 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%) (Fig. 2b). For the chronic exposure scenario (33.5 g soil, 30 days) in soil, exceedance for clothianidin in soil was above 5% for all exposure endpoints in *Cucurbita* crops (geometric mean honey bee $LC_{50} = 35.8\%$; lowest honey bee $LC_{50} = 44.3\%$; solitary bee surrogate $LC_{50} = 68.7\%$) (Fig. 3a). For imidacloprid, exceedance for all lethal endpoints was also high under the chronic exposure scenario (geometric mean honey bee $LC_{50} = 39.8\%$; lowest honey bee $LC_{50} = 57.8\%$; solitary bee surrogate $LC_{50} = 85.4\%$) (Fig. 3b). Exceedance under both the acute and chronic exposure scenarios for chlorantraniliprole in soil was below 5% for all lethal dose endpoints assessed (Fig. 3c). Because high limits of detection and quantification (LOD/LOQ) rendered no quantifiable residue samples, EEDs could not be fitted to data for thiamethoxam.

Ground-Nesting Bees in Field Crops. Clothianidin was detected in 96.34%, imidacloprid was detected in 10.97%, and thiamethoxam was detected in 81.48% of soil samples taken from Ontario field crops ($n = 82$)[13]. Hoary squash bee exposure amounts (2.23 g soil-acute exposure; 33.5 g soil-chronic exposure) were used as a surrogate for exposure to determine exceedance for ground-nesting bees generally. In the acute exposure scenario no endpoint for imidacloprid (Fig. S1) showed exceedance above 5%, and only the solitary bee surrogate LC_{50} endpoint showed exceedance above 5% for thiamethoxam (25.6%: Fig. 4b). In contrast, for clothianidin the probability of exceedance was greater than 5% for all exposure endpoints (honey bee geometric mean $LC_{50} = 11.72\%$; honey bee lowest $LC_{50} = 27.25\%$; solitary bee surrogate $LC_{50} = 81.85\%$), suggesting that the risk to ground-nesting bees is high from clothianidin in field crops soils, even when exposure is acute (Fig. 4a). In the chronic scenario, probability of exceedance for clothianidin was very high for all exposure endpoints (honey bee geometric mean $LC_{50} = 87.68\%$; lowest honey bee $LC_{50} = 92.4\%$; solitary bee surrogate $LC_{50} = 98.82\%$; Fig. 5a). For thiamethoxam, probability of exceedance was also high (honey bee geometric mean $LC_{50} = 35.7\%$; honey bee lowest $LC_{50} = 37.36\%$; solitary bee surrogate $LC_{50} = 78.42\%$; Fig. 5b). Probability of exceedance for imidacloprid under the chronic exposure scenario was below 5% for the honey bee geometric mean LC_{50} (4.16%) but exceeded 5% for the honey bee lowest LC_{50} (5.89%) and the solitary bee surrogate LC_{50} (9.24%) (Fig. S2; Table S6).

Discussion

Comparing the three exposure matrices, soil had the greatest number of neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) detected, whereas imidacloprid was the only neonicotinoid detected in pollen and nectar. The maximum concentration of imidacloprid detected in soil (41.6 ng/g) was substantially greater than in pollen (4.3 ng/g) or nectar (1.1 ng/g), because imidacloprid is applied directly to soil and can bind to soil particles[31] (Table S3). Although thiamethoxam is more soluble than imidacloprid, it is quickly metabolized to

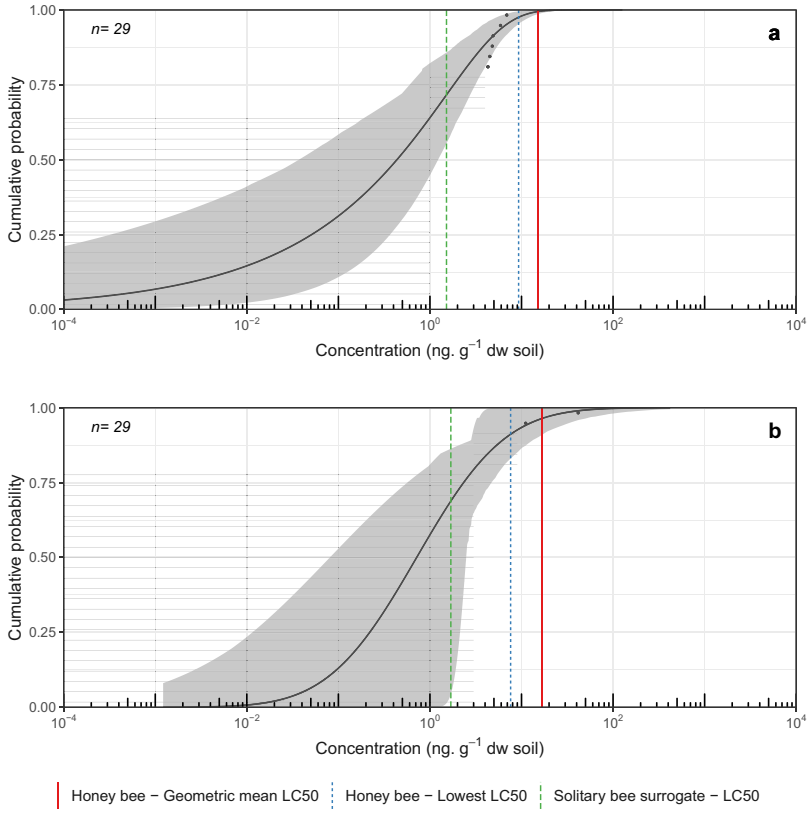

Figure 2. Environmental Exposure Distribution (EED) for acute exposure to (**a**) clothianidin and (**b**) imidacloprid concentrations measured in soil samples taken from 0–15 cm depth in *Cucurbita*-crop fields in Ontario, 2016. Effects benchmark concentrations for acute exposure (48 h, 2.23 g soil) for the hoary squash bee, *Peponapis pruinosa* (i.e. honey bee geometric mean LC_{50} = red vertical line, honey bee lowest LC_{50} = blue vertical dotted line, and the solitary bee surrogate LC_{50} = green vertical dashed line), are represented on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from one. Grey horizontal lines represent individual soil samples below the analytical limits of detection or quantification, and black dots indicate samples for which insecticide residues could be quantified. The black line indicates the line of best fit to the data via maximum likelihood estimation (MLE) with associated 95% confidence intervals (grey shading).

clothianidin[6,7,32], which is much less soluble than either imidacloprid or thiamethoxam (Table S4). Dively and Kamel[10] reported higher imidacloprid residue concentrations (60.9 ng/g for pollen, 7.4 ng/g for nectar) and greater frequencies of detection (92% of pollen samples; 88% of nectar samples) in the pollen and nectar of *Cucurbita* crops. However, the interval between application and sampling was shorter in their study (5 weeks) than in our study (8 weeks). Stoner and Eitzer[11] also reported higher concentrations of imidacloprid and thiamethoxam in *Cucurbita* pollen and nectar than found here, likely because they included anther and nectary tissue in their samples.

The analysis of the hazard of pesticides to hoary squash bees in *Cucurbita* crops is based on LD_{50}/LC_{50} for adult honey bees as this represents current regulatory standards for testing toxicity in bees[33]. It is possible that differences in sensitivity exist between these two species as not all bee species are equally sensitive[34–36]. Neonicotinoids are often more toxic to bees when exposure is via ingestion[20,21,37,38], and solitary bees are more sensitive than either honey bees or bumble bees to oral exposure[35,36,38], therefore using oral honey bee LD_{50} values in this study may under-represent oral toxicity to hoary squash bees. Toxicity via contact exposure varies much less among species[34,39], thus honey bee contact LC_{50} values may adequately represent contact toxicity for adult female hoary squash bees. The evaluation of hazard to larval squash bees using adult honey bee LD_{50} or LC_{50} values in this study may underestimate hazard because of developmental stage differences in sensitivity to neonicotinoids[6,20,21,40]. Contact lethal doses are higher than oral lethal doses for the residues we detected (Table 1).

Thiamethoxam appears to pose minimal hazard because residues were only detected in a single sample at concentrations below the limit of quantification. However, an absence of thiamethoxam residues may be the result of rapid metabolization to clothianidin, which was detected in our soil samples[6,7]. Both clothianidin (HQ = 1.82) and imidacloprid (HQ = 2.76) pose a hazard in *Cucurbita* growing systems in Ontario because they are detected frequently, and their respective HQs exceed one when summed across all exposure matrices. Imidacloprid appears to be more hazardous in *Cucurbita*-crops than clothianidin based on this approach because it was found at higher concentrations in soil and was present in both nectar and pollen (Table S3). The combined hazard of

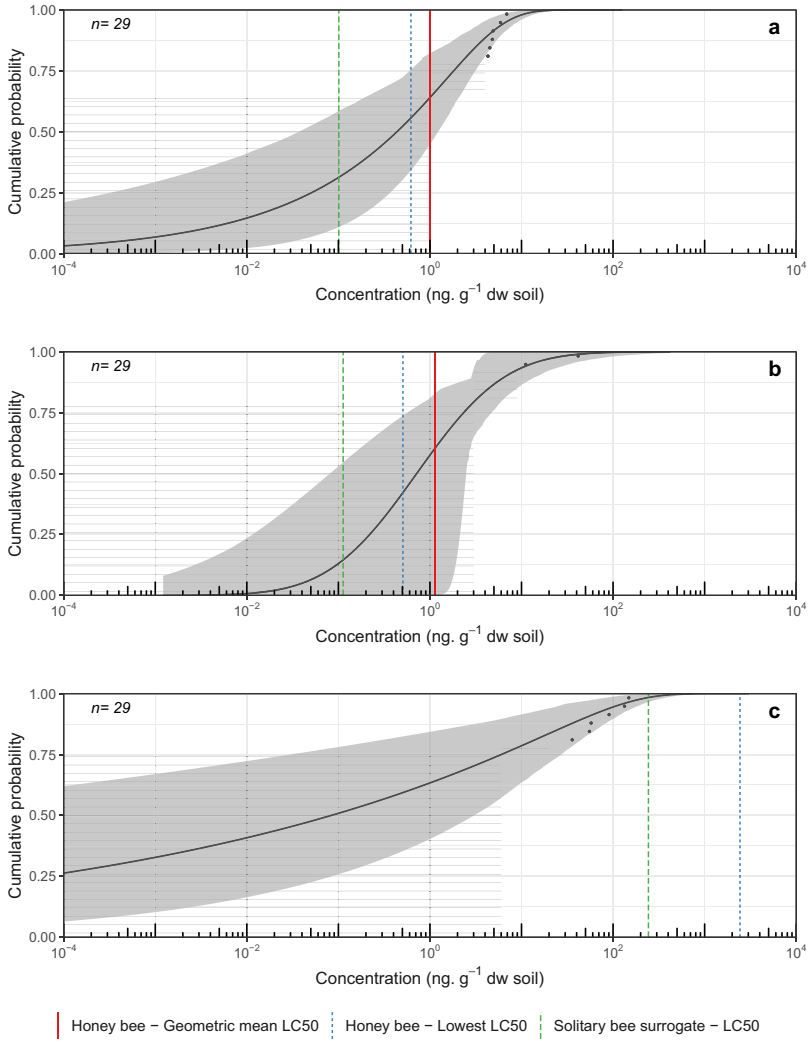

Figure 3. Environmental Exposure Distribution (EED) for chronic exposure to (**a**) clothianidin, (**b**) imidacloprid, and (**c**) chlorantraniliprole concentrations in soil samples taken from 0–15 cm depth in Cucurbita-crop fields in Ontario, 2016. Effects benchmark concentrations for chronic exposure (30 days, 33.5 g soil) for the hoary squash bee, *Peponapis pruinosa* (i.e. honey bee geometric mean LC_{50} = red vertical line, honey bee lowest LC_{50} = blue vertical dotted line, and the solitary bee surrogate LC_{50} = green vertical dashed line), are represented on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from one. Grey horizontal lines represent individual soil samples below the analytical limits of detection or quantification, and black dots indicate samples for which insecticide residues could be quantified. The black line indicates the line of best fit to the data via maximum likelihood estimation (MLE) with associated 95% confidence intervals (grey shading).

all neonicotinoids in the system was also high ($HQ_{\text{combined neonicotinoid}} = 4.59$), suggesting that hoary squash bee populations may be exposed to lethal concentrations of neonicotinoids in a worst-case scenario. Combined hazard from chlorantraniliprole in all matrices was low (HQ = 0.02), likely because it has a much higher LC_{50} than neonicotinoids.

There is common agreement that bees can be exposed to neonicotinoids from nectar and pollen[18,20,21]. Although neither pollen nor nectar were deemed hazardous here, HQs were higher for adult hoary squash bees via *Cucurbita* nectar ($HQ_{\text{nectar}} = 0.45$) than via pollen ($HQ_{\text{pollen}} = 0.03$). This was because adult exposure to pollen was considered to be contact rather than oral (as adult consumption of pollen was not evaluated), whereas adult exposure to nectar was oral and also likely an overestimation based on honey bee nectar consumption values. Although no hazard from pollen or nectar was found for the lethal dose endpoint, sublethal effects are still possible at these low HQs.

Currently, there are no studies that evaluate the risk to ground-nesting bees from direct exposure to neonicotinoids in soil, although some studies assess effects on other soil fauna[41]. Because both imidacloprid and thiamethoxam (which metabolizes to clothianidin) are applied to the soil in *Cucurbita* crops, and may persist in the soil for longer than a single growing season in Canada[13], it is unsurprising that hazard to ground-nesting hoary squash bees from neonicotinoids in soil ($HQ_{\text{soil}} = 4.32$) is much higher than even the combined hazard from

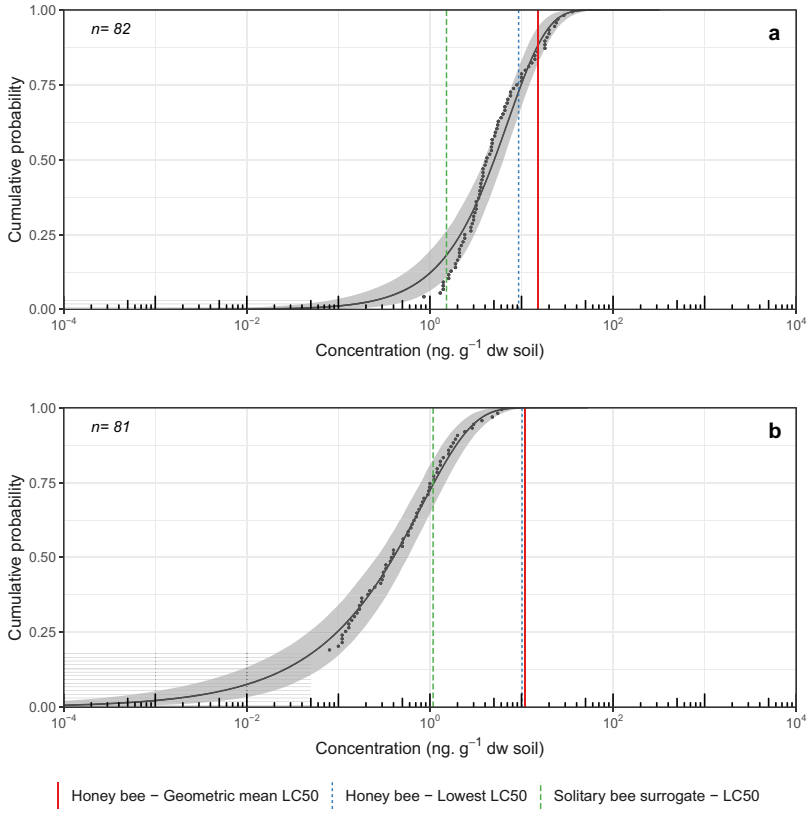

Figure 4. Environmental Exposure Distribution (EED) for acute exposure to (**a**) clothianidin and (**b**) thiamethoxam in soil from field crops (corn, soybeans, wheat) based on a government dataset[13]. Soil samples were taken from 0–15 cm depth in southern Ontario, 2016. Effects benchmark concentrations are for solitary ground-nesting bees based on the hoary squash bee (*Peponapis pruinosa*) acute exposure amounts (48 h, 2.23 g soil). Effect benchmarks (i.e. honey bee geometric mean LC_{50} = red vertical line, honey bee lowest LC_{50} = blue vertical dotted line, and the solitary bee surrogate LC_{50} = green vertical dashed line) are represented on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from one. Grey horizontal lines represent individual soil samples below the analytical limit of detection, and black dots indicate samples for which insecticide residues could be quantified. The black line indicates the line of best fit to the data via maximum likelihood estimation (MLE) with associated 95% confidence intervals (grey shading).

neonicotinoids in both pollen and nectar ($HQ_{pollen+nectar} = 0.27$; Table 1). Therefore, soil appears to be the most important route of exposure to systemic pesticides for hoary squash bees.

The combined hazard from insecticides for adult female hoary squash bees from all exposure matrices (soil, pollen, and nectar) was high, with 93% of this hazard attributable to neonicotinoids in soil (Table 1). Hoary squash bees can construct more than one nest per season when environmental conditions (e.g. nectar and pollen resources, weather) permit[17]. However, female hoary squash bees in this study were already exposed to doses above lethal levels of both imidacloprid and clothianidin (HQs > 1) during the construction of a single nest, rendering it unlikely they could construct another. Indeed, under present soil conditions in Ontario *Cucurbita* crops, pesticide exposure may preclude the construction of even a single 5-cell nest in a season, even if all other conditions are favourable. Excluding exposure from nectar, which was not evaluated, hazard to larval stages was low ($HQ_{larvae\ pollen} = 0.06$). It is reasonable to expect that hazard from nectar is also low for larval stages because their consumption of nectar is much less than for adult females as they do not undertake energy-expensive nest construction or foraging activities. For honey bees, adult pollen foragers consume 150% more nectar than larvae[42]. Here we have assumed that larval hoary squash bees are protected from direct exposure to neonicotinoids in soil by the waterproof nature of the nest cell lining[17], an assumption that requires further critical investigation.

The percentage translocation of insecticide residues from soil to bees is currently unknown. Our initial assumption was translocation at 100%, but recognizing the uncertainty around translocation rates, we also present exceedances for four alternative scenarios with lower rates (Table S8). Using a probabilistic approach for the hoary squash bee in the acute exposure scenario (48 h, 2.23 g soil; Fig. 2, Table S6) at 100% translocation, the probability of exceedance of the mean honey bee LC_{50} was below 5% for imidacloprid and clothianidin. However, for the solitary bee surrogate LC_{50} endpoint residues of both imidacloprid (31.2%) and clothianidin (28.3%) in soil exceeded 5% (Fig. 2, Table S6). In the chronic exposure scenario, the amount of exposure to soil (33.5 g soil, 30 days) was much greater, and exceedance for imidacloprid and clothianidin was greater than 5% for all lethal endpoints (Fig. 3a,b, Table S6). It is likely that at least some of the clothianidin found in *Cucurbita* crop soil is

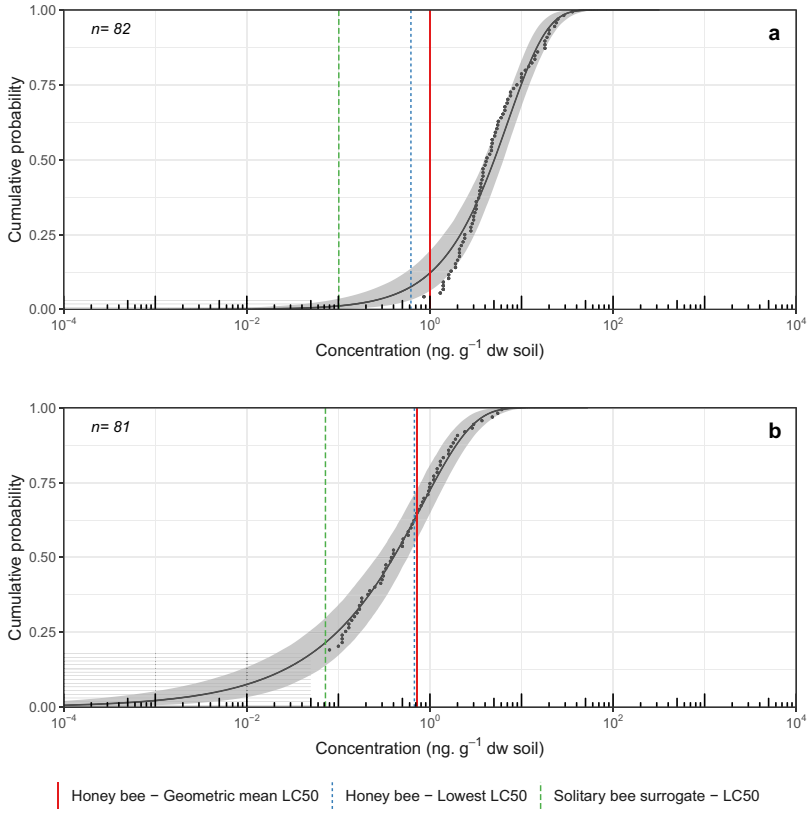

Figure 5. Environmental Exposure Distribution (EED) for chronic exposure (30 days, 33.5 g soil) to (**a**) clothianidin and (**b**) thiamethoxam in soil from field crops (corn, soybeans, wheat) based on a government dataset[13]. Soil samples were taken from 0–15 cm depth in southern Ontario, 2016. Effects benchmark concentrations are for solitary ground-nesting bees based on the hoary squash bee (*Peponapis pruinosa*) exposure model. Effect benchmarks (i.e. honey bee geometric mean LC_{50} = red vertical line, honey bee lowest LC_{50} = blue vertical dotted line, and the solitary bee surrogate LC_{50} = green vertical dashed line) are represented on the EED. Exceedance of these endpoints is calculated by subtracting the cumulative probability from one. Grey horizontal lines represent individual soil samples below the analytical limit of detection, and black dots indicate samples for which insecticide residues could be quantified. The black line indicates the line of best fit to the data via maximum likelihood estimation (MLE) with associated 95% confidence intervals (grey shading).

from the application of thiamethoxam to seeds[6]. Hilton *et al.*[7] found that ~3–46% of the residues recovered from soil sampled more than 60 days after thiamethoxam application were the metabolite clothianidin. Further work on the fate of thiamethoxam in *Cucurbita* crop fields is needed to determine whether seed-applied thiamethoxam poses a risk to hoary squash bees via its metabolite, clothianidin. Exceedance for chlorantraniliprole was not greater than 5% for any exposure endpoint in either exposure scenario, suggesting that it did not pose a risk in *Cucurbita*-crop soils in 2016 (Fig. 3c, Table S6).

There were at least three issues generating uncertainty during our assessment of risk to hoary squash bees posed by neonicotinoid residues in soil. The lack of information about insecticide toxicity for this, or indeed any other solitary ground-nesting species, is a large knowledge gap. As hoary squash bees are similar in size to honey bees, they may be well represented by the available toxicity data for honey bees, especially for contact exposure which tends to vary less among species than oral exposure[39]. Secondly, although soil-applied neonicotinoids are known to elicit negative effects on Lepidoptera pupae[43], Carabid beetles[44], Hexapoda, Collembola and Thysanoptera that live in soil[45], we could find no information on the extent to which insecticide residues in soil can pass through the cuticle or into spiracles of ground-nesting bees. We have assumed a worst-case scenario in which all the soil residues are translocated during exposure, but this is unlikely, although neonicotinoids have relatively low organic carbon-water partition coefficients (Table S4)[46]. However, even at 10% translocation, exceedances were greater than 5% for all imidacloprid endpoints, and also for the lowest honey bee LC_{50} and solitary bee surrogate LC_{50} for clothianidin, for chronic exposure in *Cucurbita* crop soils. In field crops, exceedances were greater than 5% for all endpoints for clothianidin assuming 10% translocation in a chronic exposure scenario (Table S8). Lastly, lower residue detection limits for soil are needed to align them with lethal dose concentrations for some compounds (Table S3). Confidence intervals for the EEDs in *Cucurbita*-crop soil are large due to limited sample size and high LODs. Interestingly, even at the extreme high values for confidence intervals (where exceedance would be lowest), exceedance remains above 5% for the solitary bee surrogate LC_{50} for clothianidin and imidacloprid in both acute and chronic scenarios (Figs 2 and 3). Despite its limitations, this study is significant because it is the first evaluation of risk from insecticide residues in soil for any ground-nesting bee species.

Because little information about exposure to soil for most ground-nesting bee species exists, we used hoary squash bee exposure to soil as a surrogate for other ground-nesting bees. We estimated soil exposure of 33.5 g for hoary squash bee females, which is comparable to data for some other ground-nesting bee species. For example, soil exposure for *Andrena prunorum* or *Nomia melanderi* females are estimated as 30.23 g[47] and 26.3 ± 5.7 g[48] respectively. The difference between the low-end estimate of soil excavated by *N. melanderi* (20.6 g) and hoary squash bees (33.5 g) could represent as much as 38.5% of the latter species' exposure, highlighting the potential variability in exposure via soil across species and the limitations of the hoary squash bee model. Ground-nesting bee species vary greatly in size[14], and many are much smaller than hoary squash bees. For ground-nesting solitary bees, tunnel diameter is related to bee size because bees excavate tunnels and nest cells large enough for themselves[14]. Although other solitary ground-nesting bee species may be appreciably larger or smaller than squash bees, the ratio of their body size to the volume of soil they excavate when building a nest may be similar, providing a possible future basis upon which to compare exposure for different solitary bee species. Smaller bees may have lower exposure to neonicotinoids in soil because they construct narrower tunnels and nest cells to fit their smaller bodies[14], therefore contacting lower soil volumes overall. However smaller bee species may be more sensitive to insecticide exposure[16,34,49]. Bee body size is not necessarily correlated to the depth that vertical tunnels in nests are excavated in soil[50]. Solitary bees are physiologically limited in their reproductive capacity and generally build 1–8 brood cells per nest[14]. Until more information emerges, the hoary squash bee is the best model available to evaluate risk from exposure to pesticide residues in soil for ground-nesting bees in general and provides a starting point to understand risk from insecticides residues in soil for these bees.

Neonicotinoid residues detected in Ontario agricultural soils reflect variation in usage for different crops. For *Cucurbita* crops, imidacloprid and clothianidin were the most commonly detected neonicotinoids, with a single detection of thiamethoxam at an unquantifiable concentration. For field crops, clothianidin and thiamethoxam were more commonly detected. Exposure to clothianidin residues for bees nesting in field crop soils appeared to be ubiquitous: 96.34% of soil samples taken before spring planting contained clothianidin applied in the previous season. The probabilities of exceedance were high for all clothianidin exposure endpoints for both the acute (Fig. 4) and chronic (Fig. 5) scenarios. As clothianidin-treated seeds are planted in a new cropping cycle, releasing more residues into the soil, these exceedances would likely increase. About 70% of the solitary bee species in eastern Canada nest in the ground[51], many of which are associated with agriculture, including species in the genera *Agapostemon*, *Andrena*, *Anthophora*, *Colletes*, *Eucera*, *Halictus*, *Lasioglossum*, *Megachile* and *Melissodes*[52,53]. Ground-nesting bees from 13 genera have been collected in corn and soybean fields in Iowa[54,55]. Although this does not prove that these species were nesting within fields, the small foraging ranges of solitary bees[56] suggest that many likely were. Various bees are active at different times during the season[51,57]. Those species active in the early spring may be exposed to the minimum residue concentrations described here, but those active post-planting may be exposed to much higher concentrations in soil.

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

Thiamethoxam is used as a seed treatment on both field corn and soybean crops in Ontario and was applied to 1.98 million acres (66% of treated acres) in the 2016 season[58]. For the chronic exposure scenario, the risk to ground-nesting bees from thiamethoxam was high for all exposure endpoints (Fig. 5b). For the acute exposure scenario, the risk from thiamethoxam was less than 5% for all exposure endpoints except the solitary bee surrogate LC_{50} (25.56%: Fig. 4b). The apparently lower risk to ground-nesting bees from thiamethoxam may be the product of its tendency to break down quickly into clothianidin in soil[6]. Although the risk to ground-nesting bees from acute exposure to imidacloprid in field crop soil was below the 5% threshold for all exposure endpoints (Fig. S1), exceedance rose above the threshold for the solitary bee surrogate LC_{50} (9.24%) under the chronic exposure scenario (Fig. S2). The lower risk associated with imidacloprid in field crop soil may be because it is used in only 11% of treated field crop acres[58]. One of the main concerns around neonicotinoid insecticide exposure for ground-nesting bees is their use in soil applications as treated seed. This has been partially mitigated in Ontario by increased regulation of neonicotinoid-treated corn and soybean seed[59] but has not yet been addressed for other crops.

In conclusion, neonicotinoid residues in soil pose a high risk to female hoary squash bees as they construct their nests in *Cucurbita*-crop growing systems or in field crop soils. These demonstrable risks for hoary squash bees seem likely to be applicable to other species of ground-nesting bees nesting in agricultural soils. Further work is needed to determine the relative sensitivity of the hoary squash bee to neonicotinoid exposure compared to honey bees, and to explicitly determine the extent and impacts of larval exposure in soil. Advances in analytical techniques are also needed to achieve lower limits of detection in soil that mirror lethal endpoints for solitary bees. Recognition and mitigation of risks from exposure to neonicotinoids in agricultural soil are urgently needed to protect these important crop pollinators.

Methods

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

pollen grains per anther (mean ± sd = 0.0302 ± 0.0211 g; n = 25) by the mean number of pollen grains per anther (mean ± sd = 18438 ± 9810; n = 40) (0.0302 g/18438 pollen grains = 1.64×10^{-6} g/pollen grain).

Information from the literature and this study were used to determine the realistic amount of pollen, nectar, and soil that adult female hoary squash bees would be exposed to via contact or ingestion, or that larvae would be exposed to via ingestion (Table S2). Males were not included in the evaluation, but are likely less exposed than females because they do not construct or provision nests[17]. The amount of pollen consumed by larvae (0.0542 g pollen/nest cell) was calculated by multiplying the mass of a single pollen grain (as calculated above = 1.64×10^{-6} g) by the mean number of pollen grains in hoary squash bee larval provisions (33045.4 ± 12675.2 pollen grains)[30]. Larval exposure to pesticide residues via contact with pollen in provisions was not evaluated. Contact exposure for adult females was assessed to be five times that for each larva because on average females provision five cells per nest[17]. The amount of pollen and nectar ingested by adult females is unknown. However, for pollen-collecting honey bee workers, which are most like adult female hoary squash bees in their foraging behaviour, nectar consumption has been estimated at 10.4 mg sugar/day to fly to and within foraging patches[42]. Based on this assumption, and the knowledge that *Cucurbita pepo* nectar is typically 40% sugar by volume[30], we assessed that each female hoary squash bee would consume 312 mg sugar in 780 mg of *Cucurbita* nectar to meet their energy requirements over 30 days. This is likely an over estimation as the foraging radius of squash bees from nest to flower patch is much smaller than for honey bees (honey bee foraging radius: mean = 5.5, maximum = 15 km[60]; oligolectic solitary bee foraging radius < 260 m[56]). Using hoary squash bee nest dimensions[17], the volume of soil excavated by a female bee during nest construction is 25.19 cm^3 (Table S1). Multiplying the volume by the bulk density (BD) of loam soil, a common agricultural soil (BD_{loam} = 1.33 g/cm^3)[61], gives the mass of soil that a female hoary squash bee contacts as she constructs a nest with 5 cells over 30 days (25.19 $cm^3 \times 1.33$ g/cm^3 = 33.51 g). Acute (48 h) exposure was calculated by dividing chronic exposure by 15 (33.5 g/15 = 2.23 g; Table S2).

All samples collected from *Cucurbita* farms were submitted for analysis to University of Guelph Agri-Food Laboratories (Table S3) (ISO/IEC 17025 accredited). Pesticides were extracted using the QuEChERS Method[62]. Samples were analyzed using their TOPS-142 LC pesticide screen, modified from the Canadian Food Inspection Agency (CFIA) PMR-006-V1.0 method including high performance liquid chromatography paired with electrospray ionization and tandem mass spectrometry and gas chromatography paired with tandem mass spectrometry.

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

$$Exposure\ Concentration\ Benchmark,\ (ng\ a.i.\ /g\ matrix) = \frac{Lethal\ Dose\ Effect\ Endpoint\ (ng\ a.i.\ /bee)}{Amount\ of\ soil\ exposure\ (g\ matrix/bee)}. \quad (1)$$

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

$$HQ = \frac{(Concentration\ of\ residue\ in\ matrix)(Amount\ of\ exposure\ to\ matrix)}{Honey\ bee\ LD50\ or\ LC50}. \quad (2)$$

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

EEDs were generated from the sample concentrations by fitting a log-normal or gamma distribution to the data via maximum likelihood estimation (MLE), using the fitdistrcens function in the R package fitdistrplus[69,70].

This function allows for the fit of censored (right-, left-, or interval-censored) data enabling the use of the available "non-detect" (samples containing residues below the limit of detection (LOD)), and interval (samples containing residues above the limit of detection (LOD) but below the limit of quantification (LOQ)) data. The fitdistrplus 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[69]. 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[69]. The fit to other distributions (Weibull and Exponential) was 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 data (Table S7 provides model parameters for all EEDs). For *Cucurbita* crop soils, the best fit to the data was the gamma distribution for clothianidin and chlorantraniliprole, and the log-normal distribution for imidacloprid. EEDs were not constructed for thiamethoxam as there were no quantifiable residue data. For field crops soils, the best fit for all residue data was the gamma distribution. Percent exceedance (or the probability of exposure to an exposure concentration benchmark) is calculated as 1.00 minus the cumulative probability. Exceedance lower than 5% was considered acceptable risk because it assures 95% protection of the population. Benchmarks were exposure concentrations associated with the lethal dose endpoints (geomean honey bee LC_{50}, lowest honey bee LC_{50}, solitary bee surrogate LC_{50}: Table S5) under a scenario assuming 100% translocation of residues from soil to bees (Table S6). As residue translocation from soil to bee is likely lower than 100%, exceedances assuming 10, 25, 50, 75% translocation are also presented (Table S8).

Data Availability

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

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Author Contributions

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

Additional Information

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