Monitoring neonicotinoid exposure for bees in rural and peri-urban areas of the UK during the transition from pre- to post-moratorium

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Monitoring neonicotinoid exposure for bees in rural and peri-urban areas of the UK during the transition from pre- to post-moratorium.

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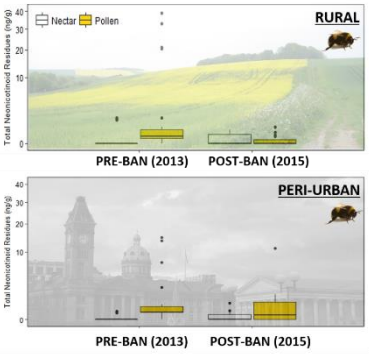
ABSTRACT: Concerns regarding the impact of neonicotinoid exposure on bee populations recently led to an EU-wide moratorium on the use of certain neonicotinoids on flowering crops. Currently evidence regarding the impact, if any, the moratorium has had on bees' exposure is limited. We sampled pollen and nectar from bumblebee colonies in rural and peri-urban habitats in three UK regions; Stirlingshire, Hertfordshire and Sussex. Colonies were sampled over three years; prior to the ban (2013), during the initial implementation when some seed-treated winter-sown oilseed rape was still grown (2014), and following the ban (2015). To compare species-level differences, in 2014 only, honeybee colonies in rural habitats were also sampled. Over half of all samples were found to be contaminated (n=408), with thiamethoxam being the compound detected at the highest concentrations in honeybee- (up to 2.29 ng/g in nectar in 2014, median≤0.1 ng/g, n=79) and bumblebee-collected pollen and nectar (up to 38.77 ng/g in pollen in 2013, median ≤0.12 ng/g, n=76). Honeybees were exposed to higher concentrations of neonicotinoids than bumblebees in 2014. While neonicotinoid exposure for rural bumblebees declined post-ban (2015), suggesting a positive impact of the moratorium, the risk of neonicotinoid exposure for bumblebees in peri-urban habitats remained largely the same between 2013 and 2015.



INTRODUCTION

Neonicotinoids are the most commonly used insecticides worldwide[1]. Their systemic nature means that, following seed-application to crops such as oilseeds or cereals, neonicotinoids become incorporated into the tissues of a plant as it grows, including pollen and nectar, the main source of food for economically important pollinators, such as honeybees and bumblebees[2]. Multiple studies have raised concerns regarding the negative impacts of neonicotinoid exposure on bees[3]. Whitehorn *et al.* (2012)[4] found that exposure of bumblebees to pollen and nectar containing 6 ng/g and 0.7 ng/g of imidacloprid respectively, resulted in slower colony growth and the production of fewer new queens, relative to unexposed colonies. Other studies have observed detrimental impacts on foraging and navigation[5,6], immunity[7] and worker mortality[8]. Based on these findings, in 2013 the European Commission instated a EU-wide moratorium on the use of three types of neonicotinoid, thiamethoxam, clothianidin and imidacloprid on bee-attractive flowering crops such as oilseed rape[9]. In 2018, this ban was subsequently expanded to include all field crops [10–12].

Criticism has been levied against studies cited in support of the moratorium, mainly for using neonicotinoid concentrations purported to exceed those routinely experienced by foraging bees[13], sparking demand for further evidence as to what constitutes a 'field-realistic' dose. Several studies have screened bee-collected pollen and nectar[14–19] for neonicotinoid residues, quantifying the 'exposure landscape' by incorporating multiple chemicals from several forage sources. Concentrations have been shown to vary considerably across studies, depending on location, time of year and species. Pollen sampled from rural bumblebee colonies in Sussex, England, prior to the implementation of the moratorium in 2013, was found to contain 18 ng/g of thiamethoxam on average, with pollen collected from nests in nearby peri-urban areas containing up to 20 ng/g imidacloprid[15] (mean=6.5 ng/g), well above the 6 ng/g used by Whitehorn *et al.*[9]. A large scale Swedish field study found clothianidin concentrations averaging 5.4 ng/g in nectar sampled from bumblebees foraging in fields of seed-treated oilseed rape (range 1.4-14 ng/g)[16]. In contrast, a study conducted in Germany found considerably lower average concentrations (0.88 ng/g) in pollen collected from bumblebee nests adjacent to neonicotinoids treated winter-sown oilseed rape[20], and a more recent study conducted across the UK, Hungary and

Germany reported that concentrations detected in pollen and nectar collected by honeybees, bumblebees and the solitary bee *Osmia bicornis* rarely exceeded 1.5 ng/g[21]. The wide ranging values reported by these studies highlights the need for further data to determine the actual exposure risk, particularly for wild bees.

Here we monitored bees' risk of neonicotinoid exposure during the period from pre- to post-moratorium, by screening pollen and nectar collected from bumblebee colonies located in several regions; Sussex (2013-2015) and Hertfordshire (2014 only) in the south of England and Stirling, Scotland (2013 only) in the north of the UK. Given the total weight of neonicotinoids applied in Scotland is much lower compared to the south of England (FERA PUS STATS database[22]), we expected the exposure risk to be lowest for bees in this region. There is currently limited data on the exposure risk for wild bees from foraging on ornamental plants grown using neonicotinoids[15,23,24] and the use of neonicotinoid-based garden sprays, therefore we monitored bumblebees in both rural and peri-urban habitats (Sussex and Stirling only), the latter consisting of domestic gardens located on the outskirts of urban areas. For bees in rural areas, we expected neonicotinoid concentrations in pollen and nectar collected in 2015 to be lower than those collected in 2013, before the implementation of the moratorium. In 2014, the impact of the ban may not have fully come into effect, as any winter-sown oilseed crops would have been drilled prior to the implementation of the ban in December 2013 and therefore may still have been seed-treated with neonicotinoids. To compare species-level differences in exposure risk during this transitional year (2014), we also screened pollen and nectar from rural honeybee colonies located in Sussex and Hertfordshire.

MATERIALS AND METHODS

Site Information Bumblebee colonies (*B.terrestris audax*) were obtained from Agralan Ltd., Swindon, UK (originating from Biobest, Belgium) and in late spring (late May to early June, see Table 1 for exact dates) were placed into the field:

i) to monitor exposure risk over the course of the implementation of the ban for both rural and peri-urban habitats, bumblebee colonies were placed in rural (n=135, n=32-47/year) and peri-urban (n=42, 12-15/year) locations across Sussex each year between 2013 and 2015. While the UK granted a derogation to use neonicotinoids on oilseed rape in 2015, this was limited to a portion of East England and did not affect the study area;

ii) to assess regional differences in neonicotinoid exposure between the north and south of the UK, prior to the implementation of the ban (2013), bumblebee colonies were also placed in rural (n=10) and peri-urban (n=20) locations in Stirling. In 2014 only, bumblebees were also placed in rural locations across Hertfordshire (n=30) for comparison with Sussex colonies;

iii) to compare species-level differences in exposure risk, 15 rural bumblebee colonies were each paired with a honeybee colony (located within 10m distance and placed into the field at the beginning of April) in both Sussex and Hertfordshire in 2014 only. Queenright honeybee colonies were obtained from experimental stocks at the University of Sussex and Rothamsted Research, which at the beginning of the experiment consisted of a single brood box and a super containing frames of fresh foundation wax, with additional space for bees to store pollen and nectar added as necessary. We also mapped which crops were grown in ten, 5 km^2 surrounding the experimental colonies in Sussex and Hertfordshire in 2014 (Fig. S4) and, where possible, asked farmers growing winter-sown oilseed rape which seed treatments they had used (Table S4).

Sampling Pollen and nectar was collected from bumblebee colonies following four, eight and ten weeks of foraging in the field. Pollen was scraped out of the colony using a stainless steel micro-spoon, which was cleaned using methanol to avoid cross-contamination. From each colony, we aimed to collect enough pollen to fill a 1.5 ml micro-centrifuge tube, to ensure enough material for chemical analysis. Concurrently, 1.5 ml of nectar was obtained from nectar pots using disposable glass pipettes. However, care was taken not to completely deplete bumblebee colony stores. Where stores were low, no sample was collected (Table 2).

For honeybees, samples were collected once per month in April, May and June 2014, with the last two sampling dates coinciding with sample collection from adjacent bumblebee colonies. Samples were obtained from freshly drawn comb, where possible, to minimise contamination from previous years. Enough pollen to fill a 1.5 ml micro-centrifuge tube was scraped out of ~10 cells using a stainless steel micro-spoon as described above, and 1.5 ml of recently stored nectar was obtained from uncapped and newly drawn comb using disposable glass pipettes. Freshness was determined by first shaking the frame to ensure nectar dripped easily out of the comb. All pollen and nectar samples were stored in individually labelled tubes and put on ice during transport back to the lab, and were then frozen at -20°C until residue analysis was performed.

Chemical analyses: Pollen and nectar samples were extracted using the QuEChERS method[14] and screened for five neonicotinoids: thiamethoxam (TMX), clothianidin (CLO), imidacloprid (IMC), acetamiprid (ACT) and thiacloprid (THC), using ultra high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). Pollen samples collected in Sussex in 2013 were not screened for acetamiprid.

Sample preparation: Pollen samples were extracted as described by Botias *et al.* (2015)[14]. Briefly, 100 mg of pollen was weighed into an Eppendorf tube and 400 pg of deuterated pesticides in ACN were added. The extraction was performed by the addition of 400 µl of water, 500 µl of ACN, 125 mg of magnesium sulphate: sodium acetate mix (4:1) and 125 mg of PSA/C18/ENVI-Carb for the dispersive solid phase extraction (dSPE) step (QuEChERS method). After the first extraction, the aqueous phase and re-suspended pellet were extracted again with 400 µl of ACN and the supernatants combined. Extracts were mixed with PSA/C18/ENVI-Carb and centrifuged. The supernatant was evaporated to dryness under vacuum, reconstituted with 120 µl ACN:H_2O (10:90) and spin filtered (0.22 µm).

Nectar samples were centrifuged at 13,000 relative centrifugal force (RCF) for 10 min to remove plant debris and the supernatant transferred into a clean eppendorf tube. Nectar samples were very viscous and were therefore weighed for more accuracy (175 ± 50 mg depending on availability) and the volume then increased to 400 µl with water. Four hundred pg of deuterated pesticide standard

mixture was added to the nectar and the samples were extracted using the same QuEChERS method described for pollen.

UHPLC-MS/MS analyses. The ultra high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method described by Botias *et al.* (2015)[14] was used for the analysis of samples. UHPLC-MS/MS analyses were carried out using a Waters Acquity UHPLC system coupled to a Quattro Premier triple quadrupole mass spectrometer from Micromass (Waters, Manchester, UK). Data were acquired using MassLynx 4.1 and the quantification was carried out by calculating the response factor of neonicotinoid compounds to their respective internal standards. Concentrations were determined using a least-square linear regression analysis of the peak area ratio *versus* the concentration ratio (native to deuterated). Method detection and quantification limits (MDL and MQL, respectively) as well as recoveries were determined as described by Botias *et al.* (2015)[14] (Table S1-3).

Quality control. One blank workup sample (*i.e.* solvent without matrix) per batch of eleven samples was included and injected on the UHPLC-MS/MS to ensure that no contamination occurred during the sample preparation. Solvent samples were also injected between sample batches to ensure that there was no carryover in the UHPLC system that might affect adjacent results in analytical runs. Samples were analysed in a random order and quality control samples (*i.e.* standards) were injected during runs every ten samples to check the sensitivity of the machine. Identities of detected neonicotinoids were confirmed by comparing ratio of MRM transitions in samples and pure standards.

Statistical Analysis. All analyses were performed using *R*-3.3.3. Residue concentrations that were above the MDL but below the MQL were assigned the MDL (Tables 2-3, range 0.03-0.10 ng/g). Concentrations below the MDL were assumed to be zero[14]. Shapiro-Wilk tests, combined with inspection of *q-q* plots, confirmed that residue data were not normally distributed. Therefore we compared the frequency of neonicotinoid contamination using contingency tables and either χ^2 or Fisher's exact tests (where expected frequencies were <5). To compare total neonicotinoid concentrations between regions (Sussex *vs.* Stirling; Sussex *vs.* Herts), habitats (Rural *vs.* Peri-Urban) and years of the study (2013 *vs.* 2015) we used non-parametric Mann-Whitney tests. For honeybee data, where frequencies of contamination and residue concentrations were compared between samples from

the same hive over several months, we used Cochran's Q test (with McNemar's test for post-hoc comparisons) and the Wilcoxon Signed-Rank test, with Bonferroni corrections to account for multiple comparisons. Given the relatively small number of pollen and nectar samples collected from each bumblebee colony, for analyses involving bumblebees we pooled samples collected after four and eight weeks in the field.

RESULTS

Bumblebees: In total, 233 pollen and nectar samples were collected from bumblebee colonies placed in rural and peri-urban habitats in the regions of Stirling, Sussex and Hertfordshire between 2013 and 2015. Forty percent of all samples screened were found to be contaminated with neonicotinoids, predominantly thiamethoxam (23%), thiacloprid (15%) and imidacloprid (10%). Pollen samples were more often contaminated (62% samples) than nectar (25% samples) and the mean combined total residues detected in pollen (Pollen N=132, 62% samples, mean± standard deviation (SD) =1.44±5.44 ng/g, median <MDL, max= 38.77 ng/g) were more than ten times higher (Nectar N=101, mean± SD= 0.12±0.44 ng/g, median <MDL, max=3.58 ng/g).

Differences in exposure by habitat and year: In 2013, the frequency of neonicotinoid contamination was similar for pollen (Table 1, χ^2_1=0, p=1.000, Rural =58%; Peri-urban= 59%) and nectar (χ^2_1=0, p=1.000, Rural=14%, Peri-urban =14%) sampled from peri-urban (PU) and rural (R) bumblebee colonies across the regions of Sussex (SU) and Stirling (ST) (Table 1). Concentrations of neonicotinoids were very similar in nectar (Mann-Whitney $U_{21,\ 21}$=225, p=0.867, $\text{mean}_{PU}\leq 0.10$, $\text{median}_{PU}\leq 0.10$, mean_{R}±SD=0.22±0.55 ng/g, median_{R} <MDL), and though higher in pollen from rural colonies, this difference was not significant ($U_{36,\ 32}$=603.5, p=0.73; mean_{R}=3.37±9.36 ng/g, $\text{median}_{R}\leq 0.12$, mean_{PU}= 1.28±3.62 ng/g, $\text{median}_{PU}\leq 0.12$). While nectar from both habitats contained only one type of neonicotinoid, predominantly thiamethoxam, over a quarter of pollen samples from bumblebee colonies in rural (28%) and peri-urban (26%) habitats contained more than one residue. Thiamethoxam (up to 38.77 ng/g, median <0.12, mean±SD= 2.08±7.47 ng/g) and clothianidin (up to 2.08 ng/g, mean ≤0.12 ng/g, median <0.12 ng/g) were present at the highest concentrations in rural colonies. While thiamethoxam was also present in a high percentage of pollen samples collected from

peri-urban colonies in Sussex (79% samples), thiacloprid was found at the highest concentration in these samples (up to 14.8 ng/g, mean ≤0.04 ng/g, median <0.04 ng/g).

In 2014, less than 10% of pollen (n=13) and nectar (n=13) samples from rural bumblebee colonies in Sussex contained neonicotinoids, all thiamethoxam and below the method quantification limit, whereas a significantly higher proportion of both pollen (85%, χ^2_1=8.987, p=0.003, n=7) and nectar samples (80%, Nectar χ^2_1=6.152, p=0.013, n=5) from peri-urban nests were contaminated (N=12), frequently with multiple residues (40% nectar samples, 29% of pollen). Again, thiacloprid (up to 9.32 ng/g in pollen, mean=1.34±3.52 ng/g, median≤ 0.04 ng/g) and thiamethoxam (up to 3.48 ng/g in pollen, mean= 0.76±1.52, median=0.10 ng/g) and were detected at the highest concentrations.

In 2015, the frequency of neonicotinoid detection was similar for nectar collected from rural and peri-urban bumblebee colonies in Sussex (χ^2_1=0.158, p=0.691, Rural=47%, Peri-urban=33%) as were the concentrations present (Mann-Whitney $U_{19,\ 12}$=130.5, p=0.469, $mean_R$=0.10±0.15 ng/g, $median_R$ <MDL, $mean_{PU}$=0.08±0.17 ng/g, $median_{PU}$ <MDL). While the frequency of detection (Rural=35%, Peri-urban=64%), proportion of samples with multiple residues (Rural=9% *vs.* Peri-urban=18%) and mean concentration of neonicotinoids were higher in pollen from peri-urban nests, the difference was not significant (χ^2_1=1.238, p=0.266, $U_{22,\ 11}$= 75.5, p=0.06, $mean_R$=0.06±0.14 ng/g, $median_R$ <MDL, $mean_{PU}$=1.29±3.30 ng/g, $median_{PU}$ <MDL). Both habitats were contaminated predominantly with thiacloprid (up to 0.44 ng/g, mean±SD=0.04±0.11 ng/g, median <MDL), and imidacloprid (up to 11.16 ng/g in peri-urban nests, mean±SD=0.21±1.40 ng/g, median <0.14), though a small proportion of peri-urban samples also contained acetamiprid (4% up to 1.4 ng/g, mean≤0.03 ng/g, median <MDL).

To compare the changing risk of exposure to peri-urban and rural bees over the transitional period from pre- to post- moratorium, we compared residue concentrations in 2013 and 2015 for Sussex bumblebee colonies only. For pollen collected from rural colonies there was a significant decrease in overall combined residue concentrations between years (Mann-Whitney $U_{23,\ 22}$=385, p=0.002, $mean_{2013}$= 5.10±11.40 ng/g, median ≤0.12 ng/g, $mean_{2015}$=0.06±0.14 ng/g, median <MDL), but not for nectar ($U_{14,\ 19}$=98, p=0.134; $mean_{2013}$= 0.20±0.51 ng/g, median <MDL, $mean_{2015}$=0.10±0.15 ng/g, median <MDL).

When considering just those neonicotinoids affected by the moratorium (thiamethoxam, clothianidin and imidacloprid), the same effect is observed, with a significant decrease in residue concentrations in pollen ($U_{23, 22}$ = 389, p <0.001, $mean_{2013}$= 5.02±11.32 ng/g, median ≤0.12 ng/g, $mean_{2015}$=0.05±0.14 ng/g, median <MDL) but not nectar between 2013 and 2015 ($U_{14, 19}$= 140, p=0.676; $mean_{2013}$= 0.20±0.51 ng/g, median <MDL, $mean_{2015}$ <MDL , median <MDL). In contrast, concentrations of thiacloprid, which was unaffected by the ban, increased significantly in nectar between 2013 and 2015 ($U_{14, 19}$= 84, p= 0.013, $mean_{2013}$<MDL, median <MDL, $mean_{2015}$=0.09±0.15 ng/g, median <MDL). Concentrations of thiacloprid in pollen remained unchanged over this period ($U_{23, 22}$=267, p=0.627, $mean_{2013}$=0.08±0.31 ng/g, median <MDL, $mean_{2015}$ < MDL, median <MDL).

For peri-urban nests, there was no significant difference in overall residue concentrations in either pollen ($U_{19, 11}$=124, *p*=0.408, $mean_{2013}$= 2.11±4.56 ng/g, median=0.12 ng/g, $mean_{2015}$=1.29±0.14 ng/g, median ≤0.04 ng/g) or nectar ($U_{13, 12}$=62.5, *p*=0.276, $mean_{2013}$= 0.02±0.05 ng/g, median <MDL, $mean_{2015}$=0.08±0.17 ng/g, median <MDL), samples collected between 2013 and 2015. When considering either the banned neonicotinoids only (Pollen, $U_{19, 11}$=134.5, p=0.188; $mean_{2013}$= 0.63±1.64 ng/g, median ≤0.12, $mean_{2015}$=1.14±3.33 ng/g, median <MDL; Nectar $U_{13, 12}$= 76, p= 0.898, $mean_{2013}$<MDL, median <MDL, $mean_{2015}$<MDL, median <MDL) or thiacloprid, which was unaffected by the ban (Pollen $U_{19, 11}$ = 104, p= 1, $mean_{2013}$= 1.47±4.41 ng/g, median <MDL, $mean_{2015}$<MDL, median <MDL, Nectar $U_{13, 12}$= 58.5, p= 0.067, $mean_{2013}$<MDL, median <MDL, $mean_{2015}$=0.05±0.13 ng/g, median <MDL), again there was no difference in the concentrations detected in pollen and nectar collected from peri-urban nests between 2013 and 2015.

Regional differences in exposure In 2013, pollen collected from bumblebee colonies in Sussex (SU) was more frequently contaminated (χ^2_1=15.62, *p*<0.001, Sussex=79%; Stirling=27%), with significantly higher concentrations of neonicotinoids than pollen collected from colonies in Stirling (ST) (Mann-Whitney $U_{42,26}$=276, *p*<0.001; $mean_{SU}$±SD=3.74±9.01 ng/g, $median_{SU}$≤0.12 ng/g $mean_{ST}$±SD =0.20±0.49 ng/g, $median_{ST}$ <MDL). Nectar was contaminated at similar frequencies (Fisher's Exact Test *p*=1.00, Sussex=14%; Stirling 12.5%) and concentrations ($U_{27,15}$=200, *p*=0.931; $mean_{SU}$=0.11±0.37 ng/g, $median_{SU}$ <MDL, $mean_{ST}$=0.13±0.47 ng/g, $median_{ST}$ <MDL).

Pollen sampled from Sussex colonies was more frequently contaminated with multiple residues (Peri-urban=37%, Rural=35%) compared to Stirling samples (Peri-urban=8%, Rural=15%), and the concentrations of thiamethoxam detected in pollen were considerably higher ($mean_{SU}$=0.58±1.64 ng/g, median=0.12 ng/g *vs.* $mean_{ST} \leq 0.12$ ng/g, median <0.12 ng/g). Sussex peri-urban colonies in particular also contained higher concentrations of thiacloprid compared to Stirling ($mean_{SU}$ =1.47±4.41 ng/g median <0.03 ng/g *vs.* $mean_{ST}$= 0.07±0.22 ng/g, median <0.03 ng/g). Imidacloprid was also frequently detected in pollen from Sussex nests in 2013, but was not detected in any samples from Stirling. Clothianidin was not detected in any Sussex nests, but accounted for the highest residue concentrations detected in nests in Stirling ($mean_{ST}$= 0.16±0.58 g/g, median <MDL, max_{ST}= 2.08 ng/g).

In 2014, residues detected in pollen and nectar samples collected from bumblebee colonies placed in rural habitats in Hertfordshire (H) and Sussex (SU) were all below the limits of quantification (<0.04-0.1 ng/g). Though there was a higher frequency of contamination of both pollen (H=36%, SU=7%) and nectar (H=20%, SU= 8%) from Hertfordshire colonies, this difference was not significant (Nectar: Fisher's Exact Test p=0.560; N_{SU}=13, N_H=10; Pollen p=0.142, N_{SU}=13, N_H=11). A small proportion of pollen from Sussex (10%), and nectar from both regions was contaminated with thiamethoxam (SU=10%; H=20%). Pollen from Hertfordshire colonies also contained acetamiprid (10%) and, more frequently, thiacloprid (40%).

Honeybees: In total, 175 pollen and nectar samples were collected from honeybee hives in Sussex and Hertfordshire between April and June May 2014, with over two thirds (68%) found to be contaminated with neonicotinoids. Total residue concentrations in nectar (N= 85, mean± SD = 0.64 ± 0.84 ng/g, median=0.20 ng/g, max= 4.23 ng/g) were approximately three times the concentrations detected in pollen (N= 90, mean± SD = 0.20 ± 0.32 ng/g, median ≤0.12 ng/g, max=1.74 ng/g), with 40% of nectar samples containing more than one residue, compared to just 9% of pollen samples. Alongside thiamethoxam, which was highly prevalent in both pollen (61% of samples) and nectar (69%), clothianidin was also frequently detected in nectar collected from honeybee hives (40%), but only once in pollen (Table 2). Imidacloprid and thiacloprid were detected in a very small percentage of samples (4-5%) and acetamipirid was not detected.

Seasonal differences: Frequency of neonicotinoid detection in pollen (Cochran's Q=24.67, df=2, p<0.001) and nectar (Q=20.38, df=2, p<0.001) sampled from honeybee colonies in 2014 changed significantly across the season. The highest frequency and concentration of neonicotinoid residues were detected in April (Fig. 3), when nearly all nectar samples collected from hives in Hertfordshire (H) and Sussex (SU) were contaminated with neonicotinoids (H=100%, $mean_H$± SD =1.46±0.66 ng/g; median=1.17 ng/g; SU=93%, $mean_{SU}$=0.95 ±1.13 ng/g, median ≤0.12 ng/g). Likewise, almost all pollen samples contained neonicotinoid residues (H=80%, $mean_H$=0.41±0.47 ng/g, median ≤0.12 ng/g; SU=100%, $mean_{SU}$=0.23±0.19 ng/g, median ≤0.12 ng/g) in April.

Between April and May, there was a similar frequency of neonicotinoid detection in both pollen (April= 90%, May=73%, McNemar test, p=0.287) and nectar (April=81%, May=80% p=0.760). While the concentration of neonicotinoid residues in pollen remained the same as the previous month (Wilcoxon signed-rank test, Z_{30}=0.28, p=0.120, $mean_{April}$=0.32±0.37 ng/g, median ≤0.12 ng/g $mean_{May}$=0.22±0.33, median ≤0.12 ng/g), neonicotinoid concentrations in nectar, previously high in comparison to pollen, declined significantly between April and May (Z_{26}=0.75, p<0.001; $mean_{April}$=1.20±0.95 ng/g, median= 1.06 ng/g, $mean_{May}$=0.65±0.72, median=0.27 ng/g).

At the final sampling point in June, neonicotinoid concentrations detected in samples from both regions were below the limit of quantification, and were significantly lower than in May (Pollen Z_{30}=0.55, p=0.003; Nectar Z_{27}=0.73, p<0.001). The frequency of neonicotinoid detection in both pollen (30% samples, McNemar test, p=0.002) and nectar (34% samples, p=0.002) was also significantly lower than the previous month (Table 2)

Regional differences: While overall neonicotinoid concentrations in pollen contamination did not differ between Hertfordshire and Sussex (Mann-Whitney $U_{45, 45}$=1014, p=0.100, $mean_H$=0.23±0.36, median ≤0.12 ng/g, $mean_{SU}$= 0.17±0.27, median ≤0.12 ng/g), concentrations in nectar were significantly higher in Hertfordshire hives ($U_{44, 42}$=1301, p≤0.001, $mean_H$=0.88±0.81, median=0.75 ng/g, $mean_{SU}$=0.40±0.80 ng/g, median ≤0.10 ng/g). Crop mapping of the five 5 km^2 study areas in each region in 2014, showed that arable crops accounted for 55% of land cover in Hertfordshire (9% oilseed rape), and 32% in Sussex (5% oilseed rape, Figure S4).

297 **Species-specific differences:** A comparison of residue concentrations in pollen and nectar
298 collected from adjacent honeybee (HB) and bumblebee (BB) nests located in rural habitats in
299 Hertfordshire and Sussex revealed significantly higher concentrations of neonicotinoid exposure for
300 honeybees compared to bumblebees (Table 1, 2, $U_{18,\,18}$= 112, p=0.04; $mean_{HB}$=0.17±0.39 ng/g, median
301 <MDL, max=1.38 ng/g; $mean_{BB}$≤0.12 ng/g, median <MDL, max ≤0.12 ng/g).

302

303 **DISCUSSION**

304 In December 2013, an EU-wide moratorium on the use of certain neonicotinoids on bee-attractive
305 flowering crops was implemented by the European Commission, which in early 2018 was subsequently
306 expanded to include all field crops. To monitor bees' exposure to neonicotinoids during the intital
307 transitional period from pre- to post-ban, between 2013 and 2015 we collected more than 400 pollen
308 and nectar samples from bumblebee and honeybee colonies located in rural and peri-urban habitats in
309 three regions across the UK, finding just over half of all samples to be contaminated with
310 neonicotinoids. While combined total concentrations of neonicotinoids in pollen collected by rural
311 bumblebees declined post-ban from an average of 5.1 ng/g in 2013, to 0.06 ng/g in 2015, suggesting a
312 positive impact of the moratorium, neonicotinoid concentrations detected in samples collected from
313 peri-urban bumblebee colonies remained largely unchanged between 2013 and 2015, indicating that the
314 risk of exposure for peri-urban bees was not altered during the transitional period, and that more could
315 be done to mitigate the risk for bees foraging in such habitats.

316 Across all samples, the highest neonicotinoid residue concentrations were detected in 2013, in
317 pollen samples collected from rural bumblebee colonies in Sussex. Concentrations of up to 38.77 ng/g
318 of thiamethoxam were detected, with the average total neonicotinoid concentrations of 5.1 ng/g similar
319 to that detected by previous studies conducted prior to the moratorium[25,15,26], and within the range
320 demonstrated to have negative impacts on bumblebee physiology[27,28], foraging efficiency[29] and colony
321 growth[28]. Pre-ban (2013), the frequency of neonicotinoid contamination was extremely high for pollen
322 sampled from bumblebee colonies in both rural and peri-urban habitats in Sussex (74% and 84% of
323 pollen samples respectively, mean=3.74 ng/g). As predicted, pollen samples collected from nests near

Stirling in 2013 were contaminated to a lesser degree (23-30% of pollen samples), and with lower concentrations (mean=0.20 ng/g). This likely reflects the fact that across Scotland, neonicotinoid use in 2013/2014 was approximately four times lower than in South East England (4, 186 kg, over 78, 345 ha *vs.* 16, 820 kg, over 197,507 ha[22]), though differences in the growth season and therefore timing of neonicotinoid application between regions may also have played a role.

Pollen and nectar samples collected from honeybee colonies in 2014, post-implementation of the ban, but when any winter-sown oilseed rape may still have been seed-treated with neonicotinoids, also had a high prevalence of neonicotinoid contamination (68% samples). Contamination was highest in April when oilseed rape was flowering (93% samples), and declined throughout the season, a phenomenon observed in several earlier studies[14,15,23,30], and hypothesised to arise from temperature increases and photo-degradation of neonicotinoid residues in plant tissues as the season progresses[31]. During this early part of the year, concentrations detected in honeybee-collected nectar averaged 1.2 ng/g, close to the average maximum concentration detected in seed-treated crop nectar, as reported by Godfray *et al.*[32] (1.9 ng/g, averaged from 20 published studies). Concentrations in pollen were considerably lower (0.32 ng/g, average maximum concentration in seed-treated crop pollen=6.1 ng/g[32]), likely reflecting honeybees' preference for collecting nectar from oilseed rape. For both bumblebees and honeybees, early spring is a period when the colony might be expected to be particularly vulnerable[33,34], and levels detected in pollen were within the range known to impair honeybee foraging performance[35], immune function[7] and alter gene expression pathways[36]. Furthermore, as observed in several previous studies[15,17,18], many of the samples we screened were found to contain more than one neonicotinoid residue, which gives rise to the potential for additive or synergistic effects. Tosi et al.[17] found when screening honeybee pollen collected from multiple apiaries across Italy for 66 different pesticides, that the frequency of detection actually peaked in summer months. Though here we did not screen for the presence of other chemical classes such as fungicides, there is evidence to suggest that exposure to certain fungicides can make bees more susceptible to the adverse effects of neonicotinoids[37].

Although the concentration of neonicotinoids in pollen and nectar sampled from rural bumblebee colonies declined between 2013 and 2015, bumblebees from both rural and peri-urban habitats were nevertheless still exposed to neonicotinoids following the implementation of the ban. Indeed 47% of nectar and 36% of pollen samples collected from rural colonies in 2015 contained neonicotinoid residues, a similar frequency as observed for peri-urban nests (33% nectar, 64% pollen), albeit at lower concentrations (mean concentration detected in pollen from rural nests = 0.06 ng/g *vs.* 1.29 ng/g detected in peri-urban pollen in 2015). This echoes the findings of Woodcock *et al.*[30] who screened honey samples submitted by beekeepers across the UK, and found that while samples harvested in 2014 were more likely to be contaminated (52% samples), 22.9% of samples harvested post-ban in 2015 also contained neonicotinoids. Similarly, a worldwide study of honey contamination spanning five years between 2012 and 2016, found 75% of 198 samples to contain neonicotinoids, with the highest prevalence in honey from North America, Asia and Europe[38].

Not only did exposure to neonicotinoids change for rural bees between 2013 and 2015, so did the chemical type. Across all samples, thiamethoxam was the most frequently detected, which is unsurprising given that, prior to the moratorium, it was the active ingredient in the mostly commonly used seed dressing on oilseed rape across Great Britain. Indeed, of fifteen farmers growing winter-sown oilseed rape within a 5 km radius of our experimental bee colonies that we interviewed in 2014, twelve had used seeds dressed with a thiamethoxam-based formulation (Cruiser®). Clothianidin, a metabolite of thiamethoxam and still in use as a seed-dressing on non-flowering cereal crops, was also frequently detected in honeybee nectar (69% samples), but only once in pollen, and was rarely detected in any samples collected from bumblebee colonies. Post-ban, acetamiprid and thiacloprid, the use of which is unaffected by the moratorium, were detected more often and at higher levels than thiamethoxam. For nectar samples collected from rural bumblebee colonies, thiacloprid concentrations actually significantly increased between 2013 and 2015. Thiacloprid is an active ingredient in many bug sprays sold in garden centres, and a recent study in which ornamental 'bee-friendly' plants were screened for multiple pesticide and fungicide residues found more than 70% of plants contained neonicotinoids, with thiacloprid present in almost half[24].

Imidacloprid was detected in a moderate proportion (10%) of samples collected from bumblebee nests throughout the duration of the study. Considering that use of imidacloprid in arable farming has dramatically declined in the UK (50% and 90% decline in weight of imidacloprid applied to cereals and oilseeds respectively between 2012 and 2014, PUS Stats database, Table S6), having been replaced by thiamethoxam and clothianidin, it is somewhat concerning that it was detected to such an extent. Woodcock et al.[30] also noted that imidacloprid was present in honey samples harvested in 2014 at a rate 'disproportional to its use' and Tosi et al.[17] detected imidacloprid in 9.1% of honeybee-collected pollen sampled from multiple apiaries across Italy in 2014 at mean concentrations of 2 ng/g, raising concerns about the persistence of this chemical in agro-environments. As previously observed when screening pollen from bumblebee colonies[15] and wild bumblebees collected in peri-urban areas[23], the highest concentrations of imidacloprid were detected in peri-urban colonies, at levels up to 11.16 ng/g in 2015 (mean=1.13 ng/g). Again, this may originate from use by the horticulture industry, since screening of ornamental plants detected imidacloprid in 38% of samples[24]. An alternative, yet untested source, is the use of imidacloprid for flea control in domestic pets and as ant poison.

Honeybees in Hertfordshire were exposed to significantly higher neonicotinoid concentrations in nectar compared to Sussex honeybees, which is most likely explained by the fact that, in 2014, there was almost double the percentage cover of treated oilseed crops (9% land cover in Hertfordshire *vs.* 5% in Sussex), and generally a higher percentage of arable land cover (55%) compared to Sussex (32%).

Overall, honeybee samples had higher concentrations of neonicotinoids compared to bumblebees. This contrasts with findings from an earlier study conducted in 2013 where the reverse was found to be true[15]. However in the previous study, colonies of each species were not placed in identical locations, therefore in addition to differences in foraging range and flower preferences[39,40], colonies may simply have been in proximity to a different range of plant species. Clearly more paired sampling of both species is required to establish whether there are consistent differences in exposure.

On the basis of evidence published post-2013, the European Food Standards Agency recently concluded that neonicotinoids do indeed pose a risk to bees[41], and in 2017 the EU commission proposed extending the moratorium to include all field crops (barring permanent greenhouse crops), which was

passed by the European Union in early 2018[10–12]. Here we have shown for the first time how exposure to neonicotinoids has changed for bees foraging in rural and peri-urban areas across the UK, since the initial implementation of the moratorium on their usage in December 2013. The exposure of rural bumblebees appears to have declined post-ban, suggesting that continued limitation of their use on flowering crops could have a positive impact on the risk for bees and other pollinators in rural areas. However, exposure for peri-urban bees remains largely unaffected, presumably as a result of contaminated ornamental plants sold in garden centres and ongoing domestic usage of neonicotinoid-based bug sprays. This is concerning given the growing interest in encouraging pollinators in urban areas; more research is needed to understand the sources of exposure and find ways to reduce it.

FIGURES

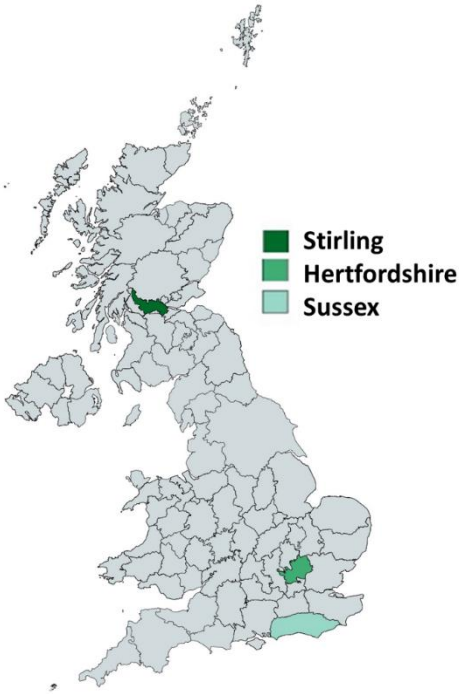


Figure 1 Map of the UK showing the regions in which honeybee (Hertfordshire and Sussex, 2014) and bumblebee (Stirling, 2013; Hertfordshire, 2014; Sussex 2013-2015) colonies were placed in rural (honeybees and bumblebees) and peri-urban (bumblebees only) habitats (see Fig. S1-3 for exact locations).

Moratorium Status	Year	Region	Bee Species	Habitat	N Colonies	Sampling Dates
Pre-ban	2013	Stirling	Bumblebee	Rural	10	12th June; 11th July; 18th July
				Peri-urban	20	6th June; 4th July; 17th July
		Sussex	Bumblebee	Rural	32	30th May; 9th June; 23rd June
				Peri-urban	12	30th May; 9th June; 23rd June
During ban (Winter-sown crops still seed-treated)	2014	Sussex	Bumblebee	Rural	47	28th May; 25th June; 9th July
				Peri-urban	15	28th May; 25th June; 9th July
			Honeybee	Rural	15	16th April; 28th May; 25th June
		Herts	Honeybee	Rural	15	16th April; 28th May; 25th June
			Bumblebee	Rural	30	28th May; 25th June; 9th July
During ban	2015	Sussex	Bumblebee	Rural	45	15th June; 13th July; 27th July
				Peri-urban	15	15th June; 13th July; 27th July

Table 1 Number of honeybee and bumblebee colonies placed in each habitat type (Peri-urban *vs.* Rural), in each region (Sussex, Stirling, Hertfordshire (Herts)) across the three years of the study (2013-2015). The specific dates colonies were sampled for pollen and nectar are listed.

						NECTAR							POLLEN							
					Method Quantification Limit (ng/g)	*0.3*	*0.3*	*0.4*	*0.08*	*0.08*			*MQL*	*0.36*	*0.36*	*0.48*	*0.12*	*0.12*		
					Method Detection Limit (ng/g)	*0.1*	*0.1*	*0.14*	*0.03*	*0.03*			*MDL*	*0.12*	*0.12*	*0.16*	*0.04*	*0.04*		
Year	**Region**	**Location**	**N Colonies**	**N Samples**	**ng/g**	**TMX**	**CLO**	**IMC**	**ACT**	**THC**	**TOTAL**	**% Multi-residue**	**N**	**TMX**	**CLO**	**IMC**	**ACT**	**THC**	**TOTAL**	**% Multi-residue**
2013	STIRLING	Peri-Urban	20	8	*Frequency %*	12.5%					**12.5%**	**0%**	13	7.7%	7.7%			15.4%	**23.1%**	**8.3%**
					Mean ±SD	≤0.10					**≤0.10**			≤0.12	≤0.12			0.06±0.22	**0.08±0.21**	
					Median	≤0.10					**≤0.10**			≤0.12	≤0.12			≤0.04	**≤0.12**	
					Max	≤0.10					**≤0.10**			≤0.12	≤0.12			0.76	**0.76**	
		Rural	10	7	*Frequency %*	12.5%					**12.5%**	**0%**	13	7.7%	7.7%			30.8%	**30.8%**	**15.3%**
					Mean ±SD	0.26± 0.68					**0.26±0.68**			≤0.12	0.16±0.58			0.15±0.36	**0.32±0.0.65**	
					Median	≤0.12					**≤0.10**			≤0.12	≤0.10			≤0.03	**≤0.12**	
					Max	1.81					**1.81**			≤0.12	2.08			1.15	**2.08**	
	SUSSEX	Peri-Urban	12	13	*Frequency %*	7.7%		7.7%			**15.4%**	**0%**	19	79%	5.26%	26.3%	*nt*	15.8%	**84.2%**	**36.8%**
					Mean ±SD	≤0.10		≤0.14			**≤0.10**			0.58±1.64	≤0.10	≤0.16		1.47±4.41	**2.11±4.56**	
					Median	≤0.10		≤0.14			**≤0.10**			≤0.12	≤0.10	≤0.16		≤0.04	**≤0.12**	
					Max	≤0.10		≤0.14			**≤0.14**			7.1	≤0.10	≤0.16		14.68	**14.8**	
		Rural	32	14	*Frequency %*	14.3%					**14.3%**	**0%**	23	60.9%	4.35%	39.1%	*nt*	17.4%	**74%**	**34.8%**
					Mean ±SD	0.2±0.51					**0.20±0.51**			4.96± 11.29	≤0.12	≤0.16		0.08±0.31	**5.10±11.41**	
					Median	≤0.10					**≤0.10**			≤0.12	≤0.12	≤0.16		≤0.04	**≤0.12**	
					Max	1.49					**1.49**			38.77	≤0.12	≤0.16		1.5	**38.93**	
2014	SUSSEX	Peri-Urban	15	5	*Frequency %*	80.0%	40.0%				**80.0%**	**40%**	7	57.1%		14.3%	14.3%	42.9%	**85.7%**	**28.6%**
					Mean ±SD	0.76±1.52	≤0.10				**0.80±1.56**			≤0.12		0.31±0.82	≤0.04	1.34±3.52	**1.73±3.43**	
					Median	0.10	≤0.10				**0.1**			≤0.12		≤0.16	≤0.04	≤0.04	**≤0.16**	
					Max	3.48	≤0.10				**3.58**			≤0.12		2.18	≤0.04	9.32	**9.32**	
		Rural	47	13	*Frequency %*	8.3%					**8.3%**	**0%**	13	7.7%					**7.7%**	**0.0%**
					Mean ±SD	≤0.10					**≤0.10**			≤0.12					**≤0.12**	
					Median	≤0.10					**≤0.10**			≤0.12					**≤0.12**	
					Max	≤0.10					**≤0.10**			≤0.12					**≤0.12**	
	HERTS	Rural	30	10	*Frequency %*	20%					**20%**	**0%**	11				9.1%	36.4%	**36.4%**	**9.1%**
					Mean ±SD	≤0.10					**≤0.10**						≤0.04	≤0.04	**≤0.04**	
					Median	≤0.10					**≤0.10**						≤0.04	≤0.04	**≤0.04**	
					Max	≤0.10					**≤0.10**						≤0.04	≤0.04	**≤0.04**	
2015	SUSSEX	Peri-Urban	15	12	*Frequency %*			16.7%		25%	**33.3%**	**8.3%**	11	9.1%		27.3%	36.35	18.2%	**63.6%**	**18.2%**
					Mean ±SD			≤0.14		0.05±0.13	**0.08±0.17**			≤0.12		1.13±3.34	0.14±0.42	≤0.04	**1.29±3.30**	
					Median			≤0.14		≤0.03	**≤0.10**			≤0.12		≤0.16	≤0.04	≤0.04	**≤0.04**	
					Max			≤0.14		0.44	**0.44**			≤0.12		11.16	1.40	≤0.04	**11.16**	
		Rural	45	19	*Frequency %*	5.3%		5.3%		36.8%	**47.4%**	**0%**	22	13.6%		9.1%	9.1%	13.6%	**36.4%**	**9.1%**
					Mean ±SD	≤0.10		≤0.14		0.09±0.15	**0.10±0.15**			≤0.12		≤0.16	≤0.04	≤0.04	**0.06±0.14**	
					Median	≤0.10		≤0.14		≤0.03	**≤0.10**			≤0.12		≤0.16	≤0.04	≤0.04	**≤0.12**	
					Max	≤0.10		≤0.14		0.42	**0.42**			≤0.12		0.60	≤0.04	≤0.04	**0.60**	

Table 2 Frequency of detection (% samples), mean (± standard deviation (SD)), median and maximum concentrations of five neonicotinoids (TMX=thiamethoxam, CLO= clothianidin, IMC= imidacloprid, ACT=acetamiprid, THC=thiacloprid) and the combined total concentration of neonicotinoids detected in pollen and nectar sampled from bumblebee colonies located in rural and peri-urban habitats in three different regions; Stirling, Hertfordshire (Herts) and Sussex. Samples were collected across three years (2013-2015). Multi-residue samples are those where more than one type of neonicotinoid was detected. *MQL*= Method quantification limit, *MDL*=Method detection limit, *nt*= not tested, ≤ less than or equal to.

				NECTAR							POLLEN							
			Method Quantification Limit (ng/g)	*0.3*	*0.3*	*0.4*	*0.08*	*0.08*			*MQL*	*0.36*	*0.36*	*0.48*	*0.12*	*0.12*		
			Method Detection Limit (ng/g)	*0.1*	*0.1*	*0.14*	*0.03*	*0.03*			*MDL*	*0.12*	*0.12*	*0.16*	*0.04*	*0.04*		
Month	**Region**	**N**		**TMX**	**CLO**	**IMC**	**ACT**	**THC**	**TOTAL**	**% Multi-residue**	**N**	**TMX**	**CLO**	**IMC**	**ACT**	**THC**	**TOTAL**	**% Multi-residue**
APRIL	**HERTS**	**15**	*Frequency of detection %*	100%	73.3%	6.7%			**100%**	**80.0%**		80%		6.6%		13.3%	**80%**	**20.0%**
			Mean ± SD (ng/g)	0.83 ± 0.48	0.63 ± 0.51	≤0.14			**1.46±0.66**		**15**	0.26±0.28		≤0.16		0.14±0.42	**0.41±0.47**	
			Median (ng/g)	0.77	0.66	≤0.14			**1.17**			0.12		≤0.16		≤0.04	**0.12**	
			Max (ng/g)	1.83	1.38	≤0.14			**1.83**			0.94		≤0.16		1.62	**1.62**	
	SUSSEX	**15**	*Frequency of detection %*	93%	47%	7%		7%	**93.3%**	**60.0%**		100%					**100%**	**0%**
			Mean ± SD (ng/g)	0.56± 0.14	0.37±0.18	≤0.14		≤0.03	**0.95 ±1.13**		**15**	0.23±0.19					**0.23±0.19**	
			Median (ng/g)	0	≤0.1	≤0.14		≤0.03	**0.58**			0.12					**0.12**	
			Max (ng/g)	1.76	2.47	≤0.03		≤0.03	**2.47**			0.6					**0.60**	
MAY	**HERTS**	**15**	*Frequency of detection %*	86.6%	73.3%				**93.3%**	**66.7%**		80%					**80%**	**0%**
			Mean ± SD (ng/g)	0.60±0.16	0.38±0.11				**1.04±0.74**		**15**	0.19±0.24					**0.19±0.24**	
			Median (ng/g)	0.45	0.10				**1.08**			0.12					**0.12**	
			Max (ng/g)	2.29	1.26				**2.29**			0.92					**0.92**	
	SUSSEX	**12**	*Frequency of detection %*	66.7%	16.7%			16.70%	**66.7%**	**25.0%**		53.3%	6.7%	6.7%		20%	**66.7%**	**20%**
			Mean ± SD (ng/g)	0.12±0.05	≤0.10			≤0.03	**0.19±0.34**		**15**	≤0.12	≤0.12	≤0.16		0.16±0.4	**0.24±0.4**	
			Median (ng/g)	0.10	≤0.10			≤0.03	**0.10**			≤0.12	≤0.12	≤0.16		≤0.04	**0.1**	
			Max (ng/g)	0.53	0.68			≤0.03	**0.68**			≤0.12	≤0.12	≤0.16		1.19	**1.2**	
JUNE	**HERTS**	**14**	*Frequency of detection %*	50%	21.4%	7.1%			**66.3%**	**21.4%**		26.7%		6.7%			**26.7%**	**8.9%**
			Mean ± SD (ng/g)	≤0.10	≤0.10	≤0.14			**0.08±0.08**		**15**	≤0.12		≤0.16			**0.09±0.26**	
			Median (ng/g)	≤0.10	≤0.10	≤0.14			**0.10**			≤0.12		≤0.16			**≤0.12**	
			Max (ng/g)	≤0.10	≤0.10	≤0.14			**≤0.14**			≤0.12		0.88			**0.88**	
	SUSSEX	**15**	*Frequency of detection %*	13.3%					**13.3%**	**0%**		26.7%		6.7%		6.7%	**33.3%**	**6.7%**
			Mean ± SD (ng/g)	≤0.10					**≤0.10**		**15**	≤0.12		≤0.16		≤0.04	**0.05±0.07**	
			Median (ng/g)	≤0.10					**≤0.10**			≤0.12		≤0.16		≤0.04	**≤0.12**	
			Max (ng/g)	≤0.10					**≤0.10**			≤0.12		≤0.16		≤0.04	**≤0.16**	

Table 3 Frequency of detection (% samples), mean (± standard deviation (SD)), median and maximum concentrations of five neonicotinoids (TMX=thiamethoxam, CLO= clothianidin, IMC= imidacloprid, ACT=acetamiprid, THC=thiacloprid) and the combined total concentration of neonicotinoids detected in honeybee nectar and pollen sampled from colonies located in in Sussex (N=15) and Hertfordshire (Herts, N=15) between April and June. Multi-residue samples are those where more than one type of neonicotinoid was detected. *MQL*= Method quantification limit, *MDL*=Method detection limit, *nt*= not tested, ≤ less than or equal to.

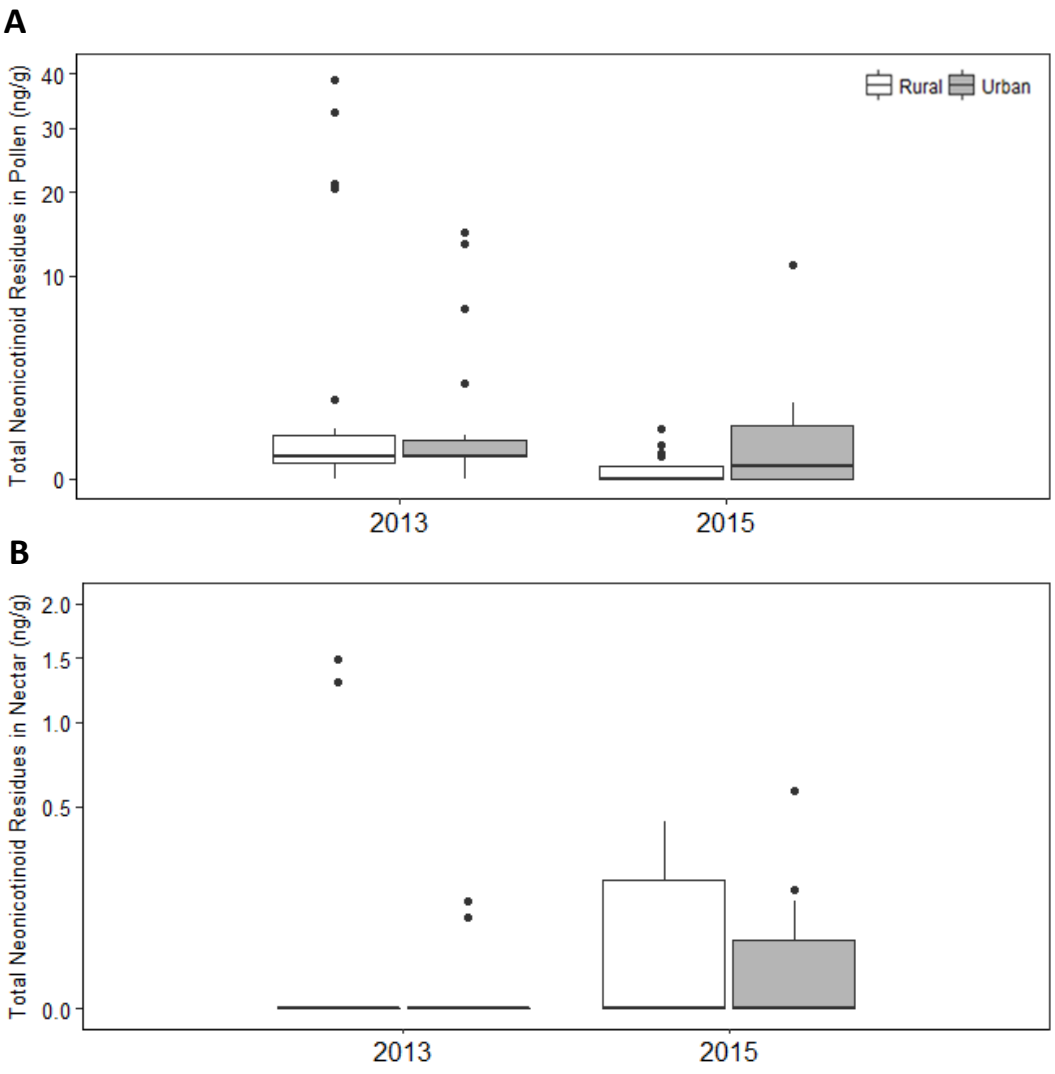


Figure 2 Total neonicotinoid concentrations (Thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid combined) detected in A) Pollen and B) Nectar samples collected from bumblebee colonies in Rural (White, N Pollen samples=45; Nectar=33) and Peri-urban (Grey, N Pollen samples= 30; Nectar=25) habitats across the region of Sussex in the years 2013 and 2015. Concentrations are plotted on a square-root scale. Black horizontal bars show median values. Box limits denote the first and third quantiles, and boxplot whiskers extend to 1.5 times the interquartile range. Outliers are represented by solid black circles.

ASSOCIATED CONTENT

Supporting Information

The following file is available free of charge.

Additional figures and tables as mentioned in the text (PDF)

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Notes

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