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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1   **Monitoring neonicotinoid exposure for bees in rural and peri-urban areas of the UK**

2   **during the transition from pre- to post-moratorium.**

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13   **ABSTRACT:** Concerns regarding the impact of neonicotinoid

14   exposure on bee populations recently led to an EU-wide

15   moratorium on the use of certain neonicotinoids on flowering

16   crops. Currently evidence regarding the impact, if any, the

17   moratorium has had on bees' exposure is limited. We sampled

18   pollen and nectar from bumblebee colonies in rural and peri-urban

19   habitats in three UK regions; Stirlingshire, Hertfordshire and Sussex. Colonies were sampled over

20   three years; prior to the ban (2013), during the initial implementation when some seed-treated winter-

21   sown oilseed rape was still grown (2014), and following the ban (2015). To compare species-level

22   differences, in 2014 only, honeybee colonies in rural habitats were also sampled. Over half of all

23   samples were found to be contaminated (n=408), with thiamethoxam being the compound detected at

24   the highest concentrations in honeybee- (up to 2.29 ng/g in nectar in 2014, median≤0.1 ng/g, n=79)

25   and bumblebee-collected pollen and nectar (up to 38.77 ng/g in pollen in 2013, median ≤0.12 ng/g,

26   n=76). Honeybees were exposed to higher concentrations of neonicotinoids than bumblebees in 2014.

27   While neonicotinoid exposure for rural bumblebees declined post-ban (2015), suggesting a positive

28   impact of the moratorium, the risk of neonicotinoid exposure for bumblebees in peri-urban habitats

29   remained largely the same between 2013 and 2015.

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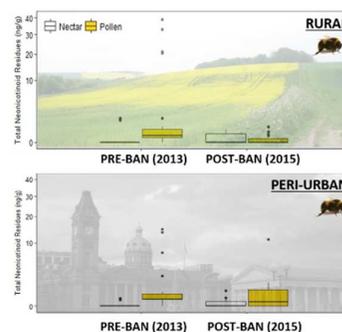
31   **INTRODUCTION**

32   Neonicotinoids are the most commonly used insecticides worldwide[1]. Their systemic nature

33   means that, following seed-application to crops such as oilseeds or cereals, neonicotinoids become

34   incorporated into the tissues of a plant as it grows, including pollen and nectar, the main source of

35   food for economically important pollinators, such as honeybees and bumblebees[2]. Multiple studies

36   have raised concerns regarding the negative impacts of neonicotinoid exposure on bees[3]. Whitehorn *et*

37   *al.* (2012)[4] found that exposure of bumblebees to pollen and nectar containing 6 ng/g and 0.7 ng/g of

38   imidacloprid respectively, resulted in slower colony growth and the production of fewer new queens,

39   relative to unexposed colonies. Other studies have observed detrimental impacts on foraging and

40   navigation[5,6], immunity[7] and worker mortality[8]. Based on these findings, in 2013 the European

41   Commission instated a EU-wide moratorium on the use of three types of neonicotinoid,

42   thiamethoxam, clothianidin and imidacloprid on bee-attractive flowering crops such as oilseed rape[9].

43   In 2018, this ban was subsequently expanded to include all field crops [10–12].

44   Criticism has been levied against studies cited in support of the moratorium, mainly for using

45   neonicotinoid concentrations purported to exceed those routinely experienced by foraging bees[13],

46   sparking demand for further evidence as to what constitutes a 'field-realistic' dose. Several studies

47   have screened bee-collected pollen and nectar[14–19] for neonicotinoid residues, quantifying the

48   'exposure landscape' by incorporating multiple chemicals from several forage sources.

49   Concentrations have been shown to vary considerably across studies, depending on location, time of

50   year and species. Pollen sampled from rural bumblebee colonies in Sussex, England, prior to the

51   implementation of the moratorium in 2013, was found to contain 18 ng/g of thiamethoxam on

52   average, with pollen collected from nests in nearby peri-urban areas containing up to 20 ng/g

53   imidacloprid[15] (mean=6.5 ng/g), well above the 6 ng/g used by Whitehorn *et al.*[9]. A large scale

54   Swedish field study found clothianidin concentrations averaging 5.4 ng/g in nectar sampled from

55   bumblebees foraging in fields of seed-treated oilseed rape (range 1.4-14 ng/g)[16]. In contrast, a study

56   conducted in Germany found considerably lower average concentrations (0.88 ng/g) in pollen

57   collected from bumblebee nests adjacent to neonicotinoids treated winter-sown oilseed rape[20], and a

58   more recent study conducted across the UK, Hungary and Germany reported that concentrations

59   detected in pollen and nectar collected by honeybees, bumblebees and the solitary bee *Osmia bicornis*

60   rarely exceeded 1.5 ng/g[21]. The wide ranging values reported by these studies highlights the need for

61   further data to determine the actual exposure risk, particularly for wild bees.

62   Here we monitored bees' risk of neonicotinoid exposure during the period from pre- to post-

63   moratorium, by screening pollen and nectar collected from bumblebee colonies located in several

64   regions; Sussex (2013-2015) and Hertfordshire (2014 only) in the south of England and Stirling,

65   Scotland (2013 only) in the north of the UK. Given the total weight of neonicotinoids applied in

66   Scotland is much lower compared to the south of England (FERA PUS STATS database[22]), we

67   expected the exposure risk to be lowest for bees in this region. There is currently limited data on the

68   exposure risk for wild bees from foraging on ornamental plants grown using neonicotinoids[15,23,24] and

69   the use of neonicotinoid-based garden sprays, therefore we monitored bumblebees in both rural and

70   peri-urban habitats (Sussex and Stirling only), the latter consisting of domestic gardens located on the

71   outskirts of urban areas. For bees in rural areas, we expected neonicotinoid concentrations in pollen

72   and nectar collected in 2015 to be lower than those collected in 2013, before the implementation of

73   the moratorium. In 2014, the impact of the ban may not have fully come into effect, as any winter-

74   sown oilseed crops would have been drilled prior to the implementation of the ban in December 2013

75   and therefore may still have been seed-treated with neonicotinoids. To compare species-level

76   differences in exposure risk during this transitional year (2014), we also screened pollen and nectar

77   from rural honeybee colonies located in Sussex and Hertfordshire.

78

79   **MATERIALS AND METHODS**

80   **Site Information** Bumblebee colonies (*B.terrestris audax*) were obtained from Agralan Ltd.,

81   Swindon, UK (originating from Biobest, Belgium) and in late spring (late May to early June, see

82   Table 1 for exact dates) were placed into the field:

83   i) to monitor exposure risk over the course of the implementation of the ban for both rural and

84   peri-urban habitats, bumblebee colonies were placed in rural (n=135, n=32-47/year) and peri-urban

85   (n=42, 12-15/year) locations across Sussex each year between 2013 and 2015. While the UK granted

86   a derogation to use neonicotinoids on oilseed rape in 2015, this was limited to a portion of East

87   England and did not affect the study area;

88   ii) to assess regional differences in neonicotinoid exposure between the north and south of the

89   UK, prior to the implementation of the ban (2013), bumblebee colonies were also placed in rural

90   (n=10) and peri-urban (n=20) locations in Stirling. In 2014 only, bumblebees were also placed in rural

91   locations across Hertfordshire (n=30) for comparison with Sussex colonies;

92   iii) to compare species-level differences in exposure risk, 15 rural bumblebee colonies were

93   each paired with a honeybee colony (located within 10m distance and placed into the field at the

94   beginning of April) in both Sussex and Hertfordshire in 2014 only. Queenright honeybee colonies

95   were obtained from experimental stocks at the University of Sussex and Rothamsted Research, which

96   at the beginning of the experiment consisted of a single brood box and a super containing frames of

97   fresh foundation wax, with additional space for bees to store pollen and nectar added as necessary.

98   We also mapped which crops were grown in ten, 5 km^2 surrounding the experimental colonies in

99   Sussex and Hertfordshire in 2014 (Fig. S4) and, where possible, asked farmers growing winter-sown

100   oilseed rape which seed treatments they had used (Table S4).

101   **Sampling** Pollen and nectar was collected from bumblebee colonies following four, eight and ten

102   weeks of foraging in the field. Pollen was scraped out of the colony using a stainless steel micro-

103   spoon, which was cleaned using methanol to avoid cross-contamination. From each colony, we aimed

104   to collect enough pollen to fill a 1.5 ml micro-centrifuge tube, to ensure enough material for chemical

105   analysis. Concurrently, 1.5 ml of nectar was obtained from nectar pots using disposable glass pipettes.

106   However, care was taken not to completely deplete bumblebee colony stores. Where stores were low,

107   no sample was collected (Table 2).

108   For honeybees, samples were collected once per month in April, May and June 2014, with the

109   last two sampling dates coinciding with sample collection from adjacent bumblebee colonies. Samples

110   were obtained from freshly drawn comb, where possible, to minimise contamination from previous

111   years. Enough pollen to fill a 1.5 ml micro-centrifuge tube was scraped out of ~10 cells using a

112   stainless steel micro-spoon as described above, and 1.5 ml of recently stored nectar was obtained from

113   uncapped and newly drawn comb using disposable glass pipettes. Freshness was determined by first

114   shaking the frame to ensure nectar dripped easily out of the comb. All pollen and nectar samples were

115   stored in individually labelled tubes and put on ice during transport back to the lab, and were then

116   frozen at   -20°C until residue analysis was performed.

117   **Chemical analyses:** Pollen and nectar samples were extracted using the QuEChERS

118   method[14] and screened for five neonicotinoids: thiamethoxam (TMX), clothianidin (CLO),

119   imidacloprid (IMC), acetamiprid (ACT) and thiacloprid (THC), using ultra high-performance liquid

120   chromatography tandem mass spectrometry (UHPLC-MS/MS). Pollen samples collected in Sussex in

121   2013 were not screened for acetamiprid.

122   **Sample preparation:** Pollen samples were extracted as described by Botias et $al.$ (2015)[14].

123   Briefly, 100 mg of pollen was weighed into an Eppendorf tube and 400 pg of deuterated pesticides in

124   ACN were added. The extraction was performed by the addition of 400 µl of water, 500 µl of ACN,

125   125 mg of magnesium sulphate: sodium acetate mix (4:1) and 125 mg of PSA/C18/ENVI-Carb for the

126   dispersive solid phase extraction (dSPE) step (QuEChERS method). After the first extraction, the

127   aqueous phase and re-suspended pellet were extracted again with 400 µl of ACN and the supernatants

128   combined. Extracts were mixed with PSA/C18/ENVI-Carb and centrifuged. The supernatant was

129   evaporated to dryness under vacuum, reconstituted with 120 µl ACN:H_2O (10:90) and spin filtered

130   (0.22 µm).

131   Nectar samples were centrifuged at 13,000 relative centrifugal force (RCF) for 10 min to

132   remove plant debris and the supernatant transferred into a clean eppendorf tube. Nectar samples were

133   very viscous and were therefore weighed for more accuracy (175 ± 50 mg depending on availability)

134   and the volume then increased to 400 µl with water. Four hundred pg of deuterated pesticide standard

135   mixture was added to the nectar and the samples were extracted using the same QuEChERS method

136   described for pollen.

137   **UHPLC-MS/MS analyses**. The ultra high-performance liquid chromatography tandem mass

138   spectrometry (UHPLC-MS/MS) method described by Botias *et al.* (2015)[14] was used for the analysis

139   of samples. UHPLC-MS/MS analyses were carried out using a Waters Acquity UHPLC system

140   coupled to a Quattro Premier triple quadrupole mass spectrometer from Micromass (Waters,

141   Manchester, UK). Data were acquired using MassLynx 4.1 and the quantification was carried out by

142   calculating the response factor of neonicotinoid compounds to their respective internal standards.

143   Concentrations were determined using a least-square linear regression analysis of the peak area ratio

144   *versus* the concentration ratio (native to deuterated). Method detection and quantification limits (MDL

145   and MQL, respectively) as well as recoveries were determined as described by Botias *et al.* (2015)[14]

146   (Table S1-3).

147   **Quality control**. One blank workup sample (*i.e.* solvent without matrix) per batch of eleven

148   samples was included and injected on the UHPLC-MS/MS to ensure that no contamination occurred

149   during the sample preparation. Solvent samples were also injected between sample batches to ensure

150   that there was no carryover in the UHPLC system that might affect adjacent results in analytical runs.

151   Samples were analysed in a random order and quality control samples (*i.e.* standards) were injected

152   during runs every ten samples to check the sensitivity of the machine. Identities of detected

153   neonicotinoids were confirmed by comparing ratio of MRM transitions in samples and pure standards.

154   **Statistical Analysis.** All analyses were performed using *R*-3.3.3. Residue concentrations that were

155   above the MDL but below the MQL were assigned the MDL (Tables 2-3, range 0.03-0.10 ng/g).

156   Concentrations below the MDL were assumed to be zero[14]. Shapiro-Wilk tests, combined with

157   inspection of *q-q* plots, confirmed that residue data were not normally distributed. Therefore we

158   compared the frequency of neonicotinoid contamination using contingency tables and either χ^2 or

159   Fisher's exact tests (where expected frequencies were <5). To compare total neonicotinoid

160   concentrations between regions (Sussex *vs.* Stirling; Sussex *vs.* Herts), habitats (Rural *vs.* Peri-Urban)

161   and years of the study (2013 *vs.* 2015) we used non-parametric Mann-Whitney tests. For honeybee

162　　data, where frequencies of contamination and residue concentrations were compared between samples

163　　from the same hive over several months, we used Cochran's Q test (with McNemar's test for post-hoc

164　　comparisons) and the Wilcoxon Signed-Rank test, with Bonferroni corrections to account for multiple

165　　comparisons. Given the relatively small number of pollen and nectar samples collected from each

166　　bumblebee colony, for analyses involving bumblebees we pooled samples collected after four and

167　　eight weeks in the field.

168　　**RESULTS**

169　　**Bumblebees:** In total, 233 pollen and nectar samples were collected from bumblebee colonies placed

170　　in rural and peri-urban habitats in the regions of Stirling, Sussex and Hertfordshire between 2013 and

171　　2015. Forty percent of all samples screened were found to be contaminated with neonicotinoids,

172　　predominantly thiamethoxam (23%), thiacloprid (15%) and imidacloprid (10%). Pollen samples were

173　　more often contaminated (62% samples) than nectar (25% samples) and the mean combined total

174　　residues detected in pollen (Pollen N=132, 62% samples, mean± standard deviation (SD) =1.44±5.44

175　　ng/g, median <MDL, max= 38.77 ng/g) were more than ten times higher (Nectar N=101, mean± SD=

176　　0.12±0.44 ng/g, median <MDL, max=3.58 ng/g).

177　　　　　**Differences in exposure by habitat and year**: In 2013, the frequency of neonicotinoid

178　　contamination was similar for pollen (Table 1, χ^2_1=0, *p*=1.000, Rural =58%; Peri-urban= 59%) and

179　　nectar (χ^2_1=0, *p*=1.000, Rural=14%, Peri-urban =14%) sampled from peri-urban (PU) and rural (R)

180　　bumblebee colonies across the regions of Sussex (SU) and Stirling (ST) (Table 1). Concentrations of

181　　neonicotinoids were very similar in nectar (Mann-Whitney $U_{21,\ 21}$=225, *p*=0.867, mean$_{PU}$≤0.10,

182　　median$_{PU}$≤0.10, mean$_R$±SD=0.22±0.55 ng/g, median$_R$ <MDL), and though higher in pollen from rural

183　　colonies, this difference was not significant ($U_{36,\ 32}$=603.5, *p*=0.73; mean$_R$=3.37±9.36 ng/g,

184　　median$_R$≤0.12, mean$_{PU}$= 1.28±3.62 ng/g, median$_{PU}$≤0.12). While nectar from both habitats contained

185　　only one type of neonicotinoid, predominantly thiamethoxam, over a quarter of pollen samples from

186　　bumblebee colonies in rural (28%) and peri-urban (26%) habitats contained more than one residue.

187　　Thiamethoxam (up to 38.77 ng/g, median <0.12, mean±SD= 2.08±7.47 ng/g) and clothianidin (up to

188　　2.08 ng/g, mean ≤0.12 ng/g, median <0.12 ng/g) were present at the highest concentrations in rural

189   colonies. While thiamethoxam was also present in a high percentage of pollen samples collected from

190   peri-urban colonies in Sussex (79% samples), thiacloprid was found at the highest concentration in

191   these samples (up to 14.8 ng/g, mean ≤0.04 ng/g, median <0.04 ng/g).

192   In 2014, less than 10% of pollen (n=13) and nectar (n=13) samples from rural bumblebee

193   colonies in Sussex contained neonicotinoids, all thiamethoxam and below the method quantification

194   limit, whereas a significantly higher proportion of both pollen (85%, χ^2_1=8.987, p=0.003, n=7) and

195   nectar samples (80%, Nectar χ^2_1=6.152, p=0.013, n=5) from peri-urban nests were contaminated

196   (N=12), frequently with multiple residues (40% nectar samples, 29% of pollen). Again, thiacloprid

197   (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

198   ng/g in pollen, mean= 0.76±1.52, median=0.10 ng/g) and were detected at the highest concentrations.

199   In 2015, the frequency of neonicotinoid detection was similar for nectar collected from rural

200   and peri-urban bumblebee colonies in Sussex (χ^2_1=0.158, p=0.691, Rural=47%, Peri-urban=33%) as

201   were the concentrations present (Mann-Whitney $U_{19,\ 12}$=130.5, p=0.469, $mean_R$=0.10±0.15 ng/g,

202     $median_R$ <MDL, $mean_{PU}$=0.08±0.17 ng/g, $median_{PU}$ <MDL). While the frequency of detection

203   (Rural=35%, Peri-urban=64%), proportion of samples with multiple residues (Rural=9% *vs.* Peri-

204   urban=18%) and mean concentration of neonicotinoids were higher in pollen from peri-urban nests,

205   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,

206     $median_R$ <MDL, $mean_{PU}$=1.29±3.30 ng/g, $median_{PU}$ <MDL). Both habitats were contaminated

207   predominantly with thiacloprid (up to 0.44 ng/g, mean±SD=0.04±0.11 ng/g, median <MDL), and

208   imidacloprid (up to 11.16 ng/g in peri-urban nests, mean±SD=0.21±1.40 ng/g, median <0.14), though

209   a small proportion of peri-urban samples also contained acetamiprid (4% up to 1.4 ng/g, mean≤0.03

210   ng/g, median <MDL).

211   To compare the changing risk of exposure to peri-urban and rural bees over the transitional period

212   from pre- to post- moratorium, we compared residue concentrations in 2013 and 2015 for Sussex

213   bumblebee colonies only. For pollen collected from rural colonies there was a significant decrease in

214   overall combined residue concentrations between years (Mann-Whitney $U_{23,\ 22}$=385, p=0.002,

215     $mean_{\mathbf{2013}}$= 5.10±11.40 ng/g, median ≤0.12 ng/g, $mean_{2015}$=0.06±0.14 ng/g, median <MDL), but not for

216 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,

217 median <MDL). When considering just those neonicotinoids affected by the moratorium

218 (thiamethoxam, clothianidin and imidacloprid), the same effect is observed, with a significant

219 decrease in residue concentrations in pollen ($U_{23, 22}$ = 389, p <0.001, mean**$_{2013}$**= 5.02±11.32 ng/g,

220 median ≤0.12 ng/g, mean$_{2015}$=0.05±0.14 ng/g, median <MDL) but not nectar between 2013 and 2015

221 ($U_{14, 19}$= 140, p=0.676; mean$_{2013}$= 0.20±0.51 ng/g, median <MDL, mean$_{2015}$ <MDL , median <MDL).

222 In contrast, concentrations of thiacloprid, which was unaffected by the ban, increased significantly in

223 nectar between 2013 and 2015 ($U_{14, 19}$= 84, p= 0.013, mean$_{2013}$<MDL, median <MDL,

224 mean$_{2015}$=0.09±0.15 ng/g, median <MDL). Concentrations of thiacloprid in pollen remained

225 unchanged over this period ($U_{23, 22}$=267, p=0.627, mean$_{2013}$=0.08±0.31 ng/g, median <MDL, mean$_{2015}$

226 < MDL, median <MDL).

227 For peri-urban nests, there was no significant difference in overall residue concentrations in

228 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

229 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,

230 mean$_{2015}$=0.08±0.17 ng/g, median <MDL), samples collected between 2013 and 2015. When

231 considering either the banned neonicotinoids only (Pollen, $U_{19, 11}$=134.5, p=0.188; mean$_{2013}$=

232 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,

233 mean$_{2013}$<MDL, median <MDL, mean$_{2015}$<MDL, median <MDL) or thiacloprid, which was

234 unaffected by the ban (Pollen $U_{19, 11}$ = 104, p= 1, mean$_{2013}$= 1.47±4.41 ng/g, median <MDL,

235 mean$_{2015}$<MDL, median <MDL, Nectar $U_{13, 12}$= 58.5, p= 0.067, mean$_{2013}$<MDL, median <MDL,

236 mean$_{2015}$=0.05±0.13 ng/g, median <MDL), again there was no difference in the concentrations

237 detected in pollen and nectar collected from peri-urban nests between 2013 and 2015.

238 **Regional differences in exposure** In 2013, pollen collected from bumblebee colonies in Sussex (SU)

239 was more frequently contaminated (χ^2_1=15.62, p<0.001, Sussex=79%; Stirling=27%), with

240 significantly higher concentrations of neonicotinoids than pollen collected from colonies in Stirling

241 (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

242 mean$_{ST}$±SD =0.20±0.49 ng/g, median$_{ST}$ <MDL). Nectar was contaminated at similar frequencies

243   (Fisher's Exact Test p=1.00, Sussex=14%; Stirling 12.5%) and concentrations ($U_{27,15}$=200, p=0.931;

244   mean$_{SU}$=0.11±0.37 ng/g, median$_{SU}$ <MDL, mean$_{ST}$=0.13±0.47 ng/g, median$_{ST}$ <MDL).

245   Pollen sampled from Sussex colonies was more frequently contaminated with multiple

246   residues (Peri-urban=37%, Rural=35%) compared to Stirling samples (Peri-urban=8%, Rural=15%),

247   and the concentrations of thiamethoxam detected in pollen were considerably higher

248   (mean$_{SU}$=0.58±1.64 ng/g, median=0.12 ng/g $vs.$ mean$_{ST}$ ≤0.12 ng/g, median <0.12 ng/g). Sussex peri-

249   urban colonies in particular also contained higher concentrations of thiacloprid compared to Stirling

250   (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).

251   Imidacloprid was also frequently detected in pollen from Sussex nests in 2013, but was not detected in

252   any samples from Stirling. Clothianidin was not detected in any Sussex nests, but accounted for the

253   highest residue concentrations detected in nests in Stirling (mean$_{ST}$= 0.16±0.58 g/g, median <MDL,

254   max$_{ST}$= 2.08 ng/g).

255   In 2014, residues detected in pollen and nectar samples collected from bumblebee colonies

256   placed in rural habitats in Hertfordshire (H) and Sussex (SU) were all below the limits of

257   quantification (<0.04-0.1 ng/g). Though there was a higher frequency of contamination of both pollen

258   (H=36%, SU=7%) and nectar (H=20%, SU= 8%) from Hertfordshire colonies, this difference was not

259   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).

260   A small proportion of pollen from Sussex (10%), and nectar from both regions was contaminated with

261   thiamethoxam (SU=10%; H=20%). Pollen from Hertfordshire colonies also contained acetamiprid

262   (10%) and, more frequently, thiacloprid (40%).

263   **Honeybees:** In total, 175 pollen and nectar samples were collected from honeybee hives in Sussex

264   and Hertfordshire between April and June May 2014, with over two thirds (68%) found to be

265   contaminated with neonicotinoids. Total residue concentrations in nectar (N= 85, mean± SD = 0.64 ±

266   0.84 ng/g, median=0.20 ng/g, max= 4.23 ng/g) were approximately three times the concentrations

267   detected in pollen (N= 90, mean± SD = 0.20 ± 0.32 ng/g, median ≤0.12 ng/g, max=1.74 ng/g), with

268   40% of nectar samples containing more than one residue, compared to just 9% of pollen samples.

269   Alongside thiamethoxam, which was highly prevalent in both pollen (61% of samples) and nectar

270 (69%), clothianidin was also frequently detected in nectar collected from honeybee hives (40%), but

271 only once in pollen (Table 2). Imidacloprid and thiacloprid were detected in a very small percentage

272 of samples (4-5%) and acetamipirid was not detected.

273 **Seasonal differences:** Frequency of neonicotinoid detection in pollen (Cochran's Q=24.67,

274 df=2, $p<0.001$) and nectar (Q=20.38, df=2, $p<0.001$) sampled from honeybee colonies in 2014

275 changed significantly across the season. The highest frequency and concentration of neonicotinoid

276 residues were detected in April (Fig. 3), when nearly all nectar samples collected from hives in

277 Hertfordshire (H) and Sussex (SU) were contaminated with neonicotinoids (H=100%, $mean_H \pm$ SD

278 =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).

279 Likewise, almost all pollen samples contained neonicotinoid residues (H=80%, $mean_H$=0.41±0.47

280 ng/g, median ≤0.12 ng/g; SU=100%, $mean_{SU}$=0.23±0.19 ng/g, median ≤0.12 ng/g) in April.

281 Between April and May, there was a similar frequency of neonicotinoid detection in both

282 pollen (April= 90%, May=73%, McNemar test, p=0.287) and nectar (April=81%, May=80%

283 p=0.760). While the concentration of neonicotinoid residues in pollen remained the same as the

284 previous month (Wilcoxon signed-rank test, Z_{30}=0.28, p=0.120, $mean_{April}$=0.32±0.37 ng/g, median

285 ≤0.12 ng/g $mean_{May}$=0.22±0.33, median ≤0.12 ng/g), neonicotinoid concentrations in nectar,

286 previously high in comparison to pollen, declined significantly between April and May (Z_{26}=0.75,

287 $p<0.001$; $mean_{April}$=1.20±0.95 ng/g, median= 1.06 ng/g, $mean_{May}$=0.65±0.72, median=0.27 ng/g).

288 At the final sampling point in June, neonicotinoid concentrations detected in samples from

289 both regions were below the limit of quantification, and were significantly lower than in May (Pollen

290 Z_{30}=0.55, p=0.003; Nectar Z_{27}=0.73, $p<0.001$). The frequency of neonicotinoid detection in both

291 pollen (30% samples, McNemar test, p=0.002) and nectar (34% samples, p=0.002) was also

292 significantly lower than the previous month (Table 2)

293 **Regional differences:** While overall neonicotinoid concentrations in pollen contamination

294 did not differ between Hertfordshire and Sussex (Mann-Whitney $U_{45, \ 45}$=1014, p=0.100,

295 $mean_H$=0.23±0.36, median ≤0.12 ng/g, $mean_{SU}$= 0.17±0.27, median ≤0.12 ng/g), concentrations in

296   nectar were significantly higher in Hertfordshire hives ($U_{44, \, 42}$=1301, $p{\le}0.001$, $mean_H$=0.88±0.81,

297   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

298   study areas in each region in 2014, showed that arable crops accounted for 55% of land cover in

299   Hertfordshire (9% oilseed rape), and 32% in Sussex (5% oilseed rape, Figure S4).

300   **Species-specific differences:** A comparison of residue concentrations in pollen and nectar

301   collected from adjacent honeybee (HB) and bumblebee (BB) nests located in rural habitats in

302   Hertfordshire and Sussex revealed significantly higher concentrations of neonicotinoid exposure for

303   honeybees compared to bumblebees (Table 1, 2, $U_{18, \, 18}$= 112, p=0.04; $mean_{HB}$=0.17±0.39 ng/g,

304   median <MDL, max=1.38 ng/g; $mean_{BB}{\le}0.12$ ng/g, median <MDL, max ≤0.12 ng/g).

305

306   **DISCUSSION**

307   In December 2013, an EU-wide moratorium on the use of certain neonicotinoids on bee-attractive

308   flowering crops was implemented by the European Commission, which in early 2018 was

309   subsequently expanded to include all field crops. To monitor bees' exposure to neonicotinoids during

310   the intital transitional period from pre- to post-ban, between 2013 and 2015 we collected more than

311   400 pollen and nectar samples from bumblebee and honeybee colonies located in rural and peri-urban

312   habitats in three regions across the UK, finding just over half of all samples to be contaminated with

313   neonicotinoids. While combined total concentrations of neonicotinoids in pollen collected by rural

314   bumblebees declined post-ban from an average of 5.1 ng/g in 2013, to 0.06 ng/g in 2015, suggesting a

315   positive impact of the moratorium, neonicotinoid concentrations detected in samples collected from

316   peri-urban bumblebee colonies remained largely unchanged between 2013 and 2015, indicating that

317   the risk of exposure for peri-urban bees was not altered during the transitional period, and that more

318   could be done to mitigate the risk for bees foraging in such habitats.

319   Across all samples, the highest neonicotinoid residue concentrations were detected in 2013, in

320   pollen samples collected from rural bumblebee colonies in Sussex. Concentrations of up to 38.77 ng/g

321   of thiamethoxam were detected, with the average total neonicotinoid concentrations of 5.1 ng/g

322   similar to that detected by previous studies conducted prior to the moratorium[25,15,26], and within the

323   range demonstrated to have negative impacts on bumblebee physiology[27,28], foraging efficiency[29] and

324   colony growth[28]. Pre-ban (2013), the frequency of neonicotinoid contamination was extremely high

325   for pollen sampled from bumblebee colonies in both rural and peri-urban habitats in Sussex (74% and

326   84% of pollen samples respectively, mean=3.74 ng/g). As predicted, pollen samples collected from

327   nests near Stirling in 2013 were contaminated to a lesser degree (23-30% of pollen samples), and with

328   lower concentrations (mean=0.20 ng/g). This likely reflects the fact that across Scotland,

329   neonicotinoid use in 2013/2014 was approximately four times lower than in South East England (4,

330   186 kg, over 78, 345 ha *vs.* 16, 820 kg, over 197,507 ha[22]), though differences in the growth season

331   and therefore timing of neonicotinoid application between regions may also have played a role.

332   Pollen and nectar samples collected from honeybee colonies in 2014, post-implementation of

333   the ban, but when any winter-sown oilseed rape may still have been seed-treated with neonicotinoids,

334   also had a high prevalence of neonicotinoid contamination (68% samples). Contamination was highest

335   in April when oilseed rape was flowering (93% samples), and declined throughout the season, a

336   phenomenon observed in several earlier studies[14,15,23,30], and hypothesised to arise from temperature

337   increases and photo-degradation of neonicotinoid residues in plant tissues as the season progresses[31].

338   During this early part of the year, concentrations detected in honeybee-collected nectar averaged 1.2

339   ng/g, close to the average maximum concentration detected in seed-treated crop nectar, as reported by

340   Godfray *et al.*[32] (1.9 ng/g, averaged from 20 published studies). Concentrations in pollen were

341   considerably lower (0.32 ng/g, average maximum concentration in seed-treated crop pollen=6.1

342   ng/g[32]), likely reflecting honeybees' preference for collecting nectar from oilseed rape. For both

343   bumblebees and honeybees, early spring is a period when the colony might be expected to be

344   particularly vulnerable[33,34], and levels detected in pollen were within the range known to impair

345   honeybee foraging performance[35], immune function[7] and alter gene expression pathways[36].

346   Furthermore, as observed in several previous studies[15,17,18], many of the samples we screened were

347   found to contain more than one neonicotinoid residue, which gives rise to the potential for additive or

348   synergistic effects. Tosi et al.[17] found when screening honeybee pollen collected from multiple

349   apiaries across Italy for 66 different pesticides, that the frequency of detection actually peaked in

350   summer months. Though here we did not screen for the presence of other chemical classes such as

351   fungicides, there is evidence to suggest that exposure to certain fungicides can make bees more

352   susceptible to the adverse effects of neonicotinoids[37].

353   Although the concentration of neonicotinoids in pollen and nectar sampled from rural

354   bumblebee colonies declined between 2013 and 2015, bumblebees from both rural and peri-urban

355   habitats were nevertheless still exposed to neonicotinoids following the implementation of the ban.

356   Indeed 47% of nectar and 36% of pollen samples collected from rural colonies in 2015 contained

357   neonicotinoid residues, a similar frequency as observed for peri-urban nests (33% nectar, 64%

358   pollen), albeit at lower concentrations (mean concentration detected in pollen from rural nests = 0.06

359   ng/g *vs.* 1.29 ng/g detected in peri-urban pollen in 2015). This echoes the findings of Woodcock *et*

360   *al.*[30] who screened honey samples submitted by beekeepers across the UK, and found that while

361   samples harvested in 2014 were more likely to be contaminated (52% samples), 22.9% of samples

362   harvested post-ban in 2015 also contained neonicotinoids. Similarly, a worldwide study of honey

363   contamination spanning five years between 2012 and 2016, found 75% of 198 samples to contain

364   neonicotinoids, with the highest prevalence in honey from North America, Asia and Europe[38].

365   Not only did exposure to neonicotinoids change for rural bees between 2013 and 2015, so did

366   the chemical type. Across all samples, thiamethoxam was the most frequently detected, which is

367   unsurprising given that, prior to the moratorium, it was the active ingredient in the mostly commonly

368   used seed dressing on oilseed rape across Great Britain. Indeed, of fifteen farmers growing winter-

369   sown oilseed rape within a 5 km radius of our experimental bee colonies that we interviewed in 2014,

370   twelve had used seeds dressed with a thiamethoxam-based formulation (Cruiser®). Clothianidin, a

371   metabolite of thiamethoxam and still in use as a seed-dressing on non-flowering cereal crops, was also

372   frequently detected in honeybee nectar (69% samples), but only once in pollen, and was rarely

373   detected in any samples collected from bumblebee colonies. Post-ban, acetamiprid and thiacloprid,

374   the use of which is unaffected by the moratorium, were detected more often and at higher levels than

375   thiamethoxam. For nectar samples collected from rural bumblebee colonies, thiacloprid

376   concentrations actually significantly increased between 2013 and 2015. Thiacloprid is an active

377   ingredient in many bug sprays sold in garden centres, and a recent study in which ornamental 'bee-

378   friendly' plants were screened for multiple pesticide and fungicide residues found more than 70% of

379   plants contained neonicotinoids, with thiacloprid present in almost half[24].

380   Imidacloprid was detected in a moderate proportion (10%) of samples collected from

381   bumblebee nests throughout the duration of the study. Considering that use of imidacloprid in arable

382   farming has dramatically declined in the UK (50% and 90% decline in weight of imidacloprid applied

383   to cereals and oilseeds respectively between 2012 and 2014, PUS Stats database, Table S6), having

384   been replaced by thiamethoxam and clothianidin, it is somewhat concerning that it was detected to

385   such an extent. Woodcock et al.[30] also noted that imidacloprid was present in honey samples

386   harvested in 2014 at a rate 'disproportional to its use' and Tosi et al.[17] detected imidacloprid in 9.1%

387   of honeybee-collected pollen sampled from multiple apiaries across Italy in 2014 at mean

388   concentrations of 2 ng/g, raising concerns about the persistence of this chemical in agro-

389   environments. As previously observed when screening pollen from bumblebee colonies[15] and wild

390   bumblebees collected in peri-urban areas[23], the highest concentrations of imidacloprid were detected

391   in peri-urban colonies, at levels up to 11.16 ng/g in 2015 (mean=1.13 ng/g). Again, this may originate

392   from use by the horticulture industry, since screening of ornamental plants detected imidacloprid in

393   38% of samples[24]. An alternative, yet untested source, is the use of imidacloprid for flea control in

394   domestic pets and as ant poison.

395   Honeybees in Hertfordshire were exposed to significantly higher neonicotinoid concentrations

396   in nectar compared to Sussex honeybees, which is most likely explained by the fact that, in 2014,

397   there was almost double the percentage cover of treated oilseed crops (9% land cover in Hertfordshire

398   vs. 5% in Sussex), and generally a higher percentage of arable land cover (55%) compared to Sussex

399   (32%).

400   Overall, honeybee samples had higher concentrations of neonicotinoids compared to

401   bumblebees. This contrasts with findings from an earlier study conducted in 2013 where the reverse

402   was found to be true[15]. However in the previous study, colonies of each species were not placed in

403   identical locations, therefore in addition to differences in foraging range and flower preferences[39,40],

404   colonies may simply have been in proximity to a different range of plant species. Clearly more paired

405   sampling of both species is required to establish whether there are consistent differences in exposure.

406   On the basis of evidence published post-2013, the European Food Standards Agency recently

407   concluded that neonicotinoids do indeed pose a risk to bees[41], and in 2017 the EU commission

408   proposed extending the moratorium to include all field crops (barring permanent greenhouse crops),

409   which was passed by the European Union in early 2018[10–12]. Here we have shown for the first time

410   how exposure to neonicotinoids has changed for bees foraging in rural and peri-urban areas across the

411   UK, since the initial implementation of the moratorium on their usage in December 2013. The

412   exposure of rural bumblebees appears to have declined post-ban, suggesting that continued limitation

413   of their use on flowering crops could have a positive impact on the risk for bees and other pollinators

414   in rural areas. However, exposure for peri-urban bees remains largely unaffected, presumably as a

415   result of contaminated ornamental plants sold in garden centres and ongoing domestic usage of

416   neonicotinoid-based bug sprays. This is concerning given the growing interest in encouraging

417   pollinators in urban areas; more research is needed to understand the sources of exposure and find

418   ways to reduce it.

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433   **FIGURES**

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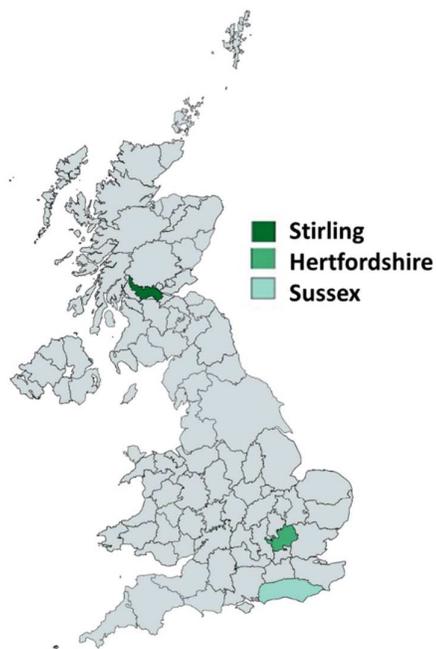
448   Figure 1 Map of the UK showing the regions in which honeybee (Hertfordshire and Sussex, 2014) and
449   bumblebee (Stirling, 2013; Hertfordshire, 2014; Sussex 2013-2015) colonies were placed in rural
450   (honeybees and bumblebees) and peri-urban (bumblebees only) habitats (see Fig. S1-3 for exact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

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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

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463 Table 1 Number of honeybee and bumblebee colonies placed in each habitat type (Peri-urban *vs.*
464 Rural), in each region (Sussex, Stirling, Hertfordshire (Herts)) across the three years of the study
465 (2013-2015). The specific dates colonies were sampled for pollen and nectar are listed.

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						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								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		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		
APRIL	SUSSEX	15								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		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								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		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		
MAY	SUSSEX	12								15								
			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		≤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								15								
			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		≤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		
JUNE	SUSSEX	15								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		≤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.

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520 **B**

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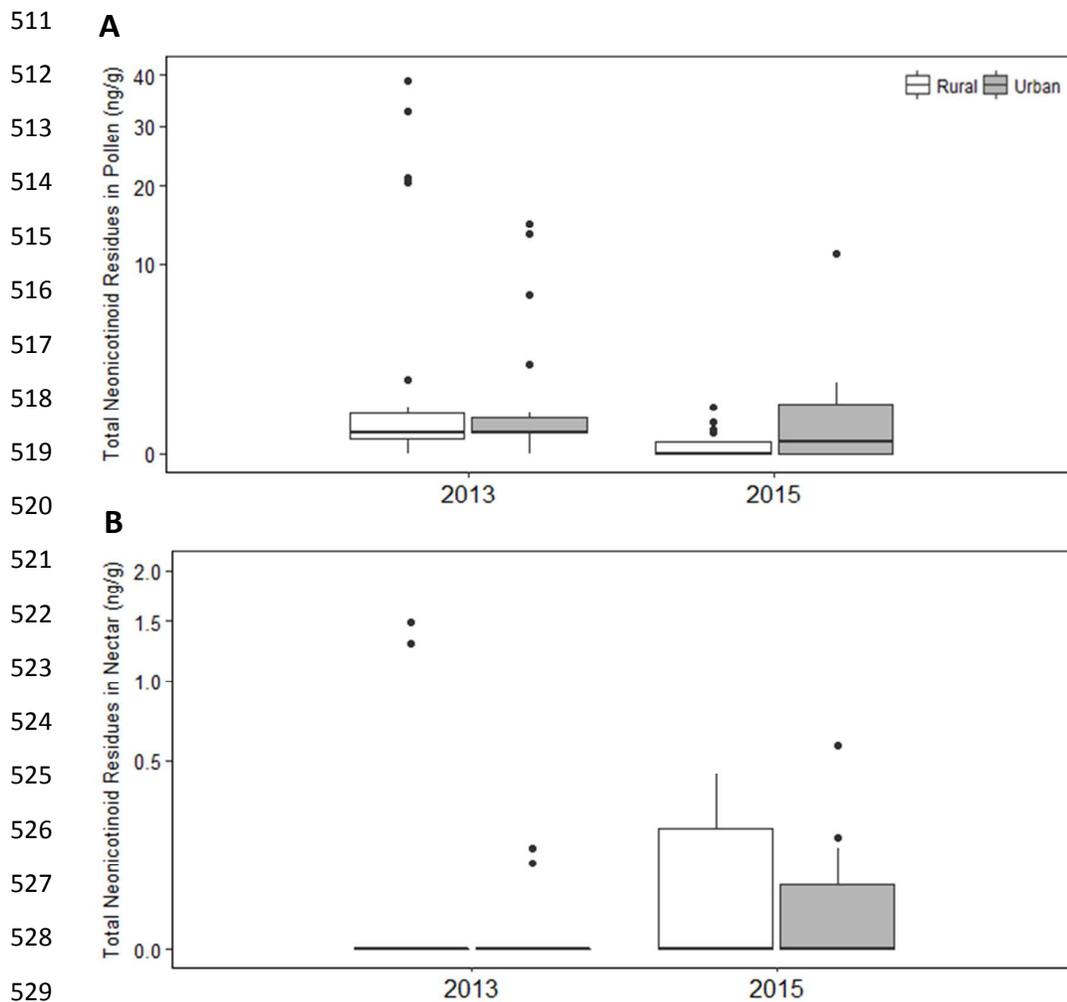
531 Figure 2 Total neonicotinoid concentrations (Thiamethoxam, clothianidin, imidacloprid, acetamiprid
532 and thiacloprid combined) detected in A) Pollen and B) Nectar samples collected from bumblebee
533 colonies in Rural (White, N Pollen samples=45; Nectar=33) and Peri-urban (Grey, N Pollen samples=
534 30; Nectar=25) habitats across the region of Sussex in the years 2013 and 2015. Concentrations are
535 plotted on a square-root scale. Black horizontal bars show median values. Box limits denote the first
536 and third quantiles, and boxplot whiskers extend to 1.5 times the interquartile range. Outliers are
537 represented by solid black circles.

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541 **ASSOCIATED CONTENT**

542 **Supporting Information**

543 The following file is available free of charge.

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

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557 **Notes**

558 The authors declare no competing financial interest.

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