



**HAL**  
open science

## Monitoring Neonicotinoid Exposure for Bees in Rural and Peri-urban Areas of the UK during the Transition from Pre- to Post-moratorium

Elizabeth Nicholls, Cristina Botias, Ellen L. Rotheray, Penelope Whitehorn, Arthur David, Robert Fowler, Thomas David, Hannah Feltham, Jennifer L. Swain, Patricia Wells, et al.

### ► To cite this version:

Elizabeth Nicholls, Cristina Botias, Ellen L. Rotheray, Penelope Whitehorn, Arthur David, et al.. Monitoring Neonicotinoid Exposure for Bees in Rural and Peri-urban Areas of the UK during the Transition from Pre- to Post-moratorium. *Environmental Science and Technology*, 2018, 52 (16), pp.9391-9402. 10.1021/acs.est.7b06573 . hal-01901001

**HAL Id: hal-01901001**

**<https://univ-rennes.hal.science/hal-01901001v1>**

Submitted on 2 Jul 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Elizabeth Nicholls, CRISTINA BOTÍAS, Ellen L Rotheray, Penelope Whitehorn, Arthur David, Robert Fowler, Thomas David, Hannah Feltham, Jennifer L. Swain, Patricia Wells, Elizabeth M Hill, Juliet L Osborne, and Dave Goulson

*Environ. Sci. Technol.*, **Just Accepted Manuscript** • DOI: 10.1021/acs.est.7b06573 • Publication Date (Web): 28 Jun 2018

Downloaded from <http://pubs.acs.org> on July 30, 2018

### Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

This document is the Accepted Manuscript version of a Published Work that appeared in final form in *Environmental Science and Technology*, copyright © American Chemical Society after peer review and technical editing by the publisher.

To access the final edited and published work see <https://doi.org/10.1021/acs.est.7b06573>



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

3 Elizabeth Nicholls<sup>a\*</sup>, Cristina Botías<sup>a‡</sup>, Ellen L. Rotheray<sup>a</sup>, Penelope Whitehorn<sup>b†</sup>, Arthur David<sup>a‡</sup>,  
4 Robert Fowler<sup>a</sup>, Thomas David<sup>a,c</sup>, Hannah Feltham<sup>b§</sup>, Jennifer L. Swain<sup>c</sup>, Patricia Wells<sup>c</sup>, Elizabeth  
5 M. Hill<sup>a</sup>, Juliet L. Osborne<sup>d</sup> & Dave Goulson<sup>a</sup>

6 <sup>a</sup>School of Life Sciences, University of Sussex, Falmer, BN1 9QG, UK

7 <sup>b</sup>School of Natural Sciences, University of Stirling, Stirling, FK9 4LA, UK

8 <sup>c</sup>Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

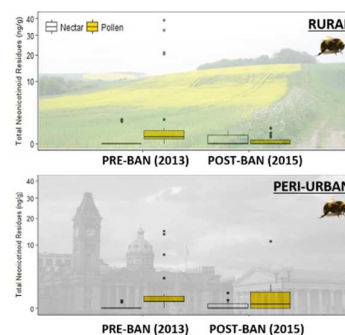
9 <sup>d</sup>Environment and Sustainability Institute, University of Exeter, Penryn, TR10 9FE, UK

10

11 [\\*e.nicholls@sussex.ac.uk](mailto:e.nicholls@sussex.ac.uk)

12

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  $\leq 0.1$  ng/g, n=79)  
25 and bumblebee-collected pollen and nectar (up to 38.77 ng/g in pollen in 2013, median  $\leq 0.12$  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.

30

## 31 INTRODUCTION

32 Neonicotinoids are the most commonly used insecticides worldwide<sup>1</sup>. 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<sup>2</sup>. Multiple studies  
36 have raised concerns regarding the negative impacts of neonicotinoid exposure on bees<sup>3</sup>. Whitehorn *et al.*  
37 *(2012)*<sup>4</sup> 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<sup>5,6</sup>, immunity<sup>7</sup> and worker mortality<sup>8</sup>. 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<sup>9</sup>.  
43 In 2018, this ban was subsequently expanded to include all field crops<sup>10-12</sup>.

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<sup>13</sup>,  
46 sparking demand for further evidence as to what constitutes a ‘field-realistic’ dose. Several studies  
47 have screened bee-collected pollen and nectar<sup>14-19</sup> 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<sup>15</sup> (mean=6.5 ng/g), well above the 6 ng/g used by Whitehorn *et al.*<sup>9</sup>. 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)<sup>16</sup>. 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<sup>20</sup>, 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<sup>21</sup>. 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<sup>22</sup>), 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<sup>15,23,24</sup> 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<sup>2</sup> 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<sup>14</sup> 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)<sup>14</sup>.  
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<sub>2</sub>O (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)<sup>14</sup> 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)<sup>14</sup>  
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<sup>14</sup>. 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  $\chi^2$  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,  $\chi^2_1=0$ ,  $p=1.000$ , Rural =58%; Peri-urban= 59%) and  
179 nectar ( $\chi^2_1=0$ ,  $p=1.000$ , 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<sub>PU</sub>≤0.10,  
182 median<sub>PU</sub>≤0.10, mean<sub>R</sub>±SD=0.22±0.55 ng/g, median<sub>R</sub> <MDL), and though higher in pollen from rural  
183 colonies, this difference was not significant ( $U_{36, 32}=603.5$ ,  $p=0.73$ ; mean<sub>R</sub>=3.37±9.36 ng/g,  
184 median<sub>R</sub>≤0.12, mean<sub>PU</sub>= 1.28±3.62 ng/g, median<sub>PU</sub>≤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  $\leq 0.04$  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%,  $\chi^2_1=8.987$ ,  $p=0.003$ , n=7) and  
195 nectar samples (80%, Nectar  $\chi^2_1=6.152$ ,  $p=0.013$ , 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 $\pm$ 3.52 ng/g, median $\leq 0.04$  ng/g) and thiamethoxam (up to 3.48  
198 ng/g in pollen, mean= 0.76 $\pm$ 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 ( $\chi^2_1=0.158$ ,  $p=0.691$ , Rural=47%, Peri-urban=33%) as  
201 were the concentrations present (Mann-Whitney  $U_{19, 12}=130.5$ ,  $p=0.469$ , mean<sub>R</sub>=0.10 $\pm$ 0.15 ng/g,  
202 median<sub>R</sub>  $<$ MDL, mean<sub>PU</sub>=0.08 $\pm$ 0.17 ng/g, median<sub>PU</sub>  $<$ 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 ( $\chi^2_1=1.238$ ,  $p=0.266$ ,  $U_{22, 11}= 75.5$ ,  $p=0.06$ , mean<sub>R</sub>=0.06 $\pm$ 0.14 ng/g,  
206 median<sub>R</sub>  $<$ MDL, mean<sub>PU</sub>=1.29 $\pm$ 3.30 ng/g, median<sub>PU</sub>  $<$ MDL). Both habitats were contaminated  
207 predominantly with thiacloprid (up to 0.44 ng/g, mean $\pm$ SD=0.04 $\pm$ 0.11 ng/g, median  $<$ MDL), and  
208 imidacloprid (up to 11.16 ng/g in peri-urban nests, mean $\pm$ SD=0.21 $\pm$ 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 $\leq$ 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<sub>2013</sub>= 5.10 $\pm$ 11.40 ng/g, median  $\leq 0.12$  ng/g, mean<sub>2015</sub>=0.06 $\pm$ 0.14 ng/g, median  $<$ MDL), but not for

nectar ( $U_{14, 19}=98$ ,  $p=0.134$ ;  $\text{mean}_{2013}=0.20\pm 0.51$  ng/g, median <MDL,  $\text{mean}_{2015}=0.10\pm 0.15$  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 ( $U_{23, 22} = 389$ ,  $p < 0.001$ ,  $\text{mean}_{2013}=5.02\pm 11.32$  ng/g, median  $\leq 0.12$  ng/g,  $\text{mean}_{2015}=0.05\pm 0.14$  ng/g, median <MDL) but not nectar between 2013 and 2015 ( $U_{14, 19}=140$ ,  $p=0.676$ ;  $\text{mean}_{2013}=0.20\pm 0.51$  ng/g, median <MDL,  $\text{mean}_{2015}<\text{MDL}$ , median <MDL). In contrast, concentrations of thiacloprid, which was unaffected by the ban, increased significantly in nectar between 2013 and 2015 ( $U_{14, 19}=84$ ,  $p=0.013$ ,  $\text{mean}_{2013}<\text{MDL}$ , median <MDL,  $\text{mean}_{2015}=0.09\pm 0.15$  ng/g, median <MDL). Concentrations of thiacloprid in pollen remained unchanged over this period ( $U_{23, 22}=267$ ,  $p=0.627$ ,  $\text{mean}_{2013}=0.08\pm 0.31$  ng/g, median <MDL,  $\text{mean}_{2015}<\text{MDL}$ , median <MDL).

For peri-urban nests, there was no significant difference in overall residue concentrations in either pollen ( $U_{19, 11}=124$ ,  $p=0.408$ ,  $\text{mean}_{2013}=2.11\pm 4.56$  ng/g, median=0.12 ng/g,  $\text{mean}_{2015}=1.29\pm 0.14$  ng/g, median  $\leq 0.04$  ng/g) or nectar ( $U_{13, 12}=62.5$ ,  $p=0.276$ ,  $\text{mean}_{2013}=0.02\pm 0.05$  ng/g, median <MDL,  $\text{mean}_{2015}=0.08\pm 0.17$  ng/g, median <MDL), samples collected between 2013 and 2015. When considering either the banned neonicotinoids only (Pollen,  $U_{19, 11}=134.5$ ,  $p=0.188$ ;  $\text{mean}_{2013}=0.63\pm 1.64$  ng/g, median  $\leq 0.12$ ,  $\text{mean}_{2015}=1.14\pm 3.33$  ng/g, median <MDL; Nectar  $U_{13, 12}=76$ ,  $p=0.898$ ,  $\text{mean}_{2013}<\text{MDL}$ , median <MDL,  $\text{mean}_{2015}<\text{MDL}$ , median <MDL) or thiacloprid, which was unaffected by the ban (Pollen  $U_{19, 11}=104$ ,  $p=1$ ,  $\text{mean}_{2013}=1.47\pm 4.41$  ng/g, median <MDL,  $\text{mean}_{2015}<\text{MDL}$ , median <MDL, Nectar  $U_{13, 12}=58.5$ ,  $p=0.067$ ,  $\text{mean}_{2013}<\text{MDL}$ , median <MDL,  $\text{mean}_{2015}=0.05\pm 0.13$  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 ( $\chi^2_1=15.62$ ,  $p<0.001$ , Sussex=79%; Stirling=27%), with significantly higher concentrations of neonicotinoids than pollen collected from colonies in Stirling (ST) (Mann-Whitney  $U_{42,26}=276$ ,  $p<0.001$ ;  $\text{mean}_{\text{SU}}\pm\text{SD}=3.74\pm 9.01$  ng/g,  $\text{median}_{\text{SU}}\leq 0.12$  ng/g,  $\text{mean}_{\text{ST}}\pm\text{SD}=0.20\pm 0.49$  ng/g,  $\text{median}_{\text{ST}}<\text{MDL}$ ). 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  $\text{mean}_{\text{SU}}=0.11\pm 0.37$  ng/g,  $\text{median}_{\text{SU}} < \text{MDL}$ ,  $\text{mean}_{\text{ST}}=0.13\pm 0.47$  ng/g,  $\text{median}_{\text{ST}} < \text{MDL}$ ).

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 ( $\text{mean}_{\text{SU}}=0.58\pm 1.64$  ng/g,  $\text{median}=0.12$  ng/g vs.  $\text{mean}_{\text{ST}}\leq 0.12$  ng/g,  $\text{median} < 0.12$  ng/g). Sussex peri-  
249 urban colonies in particular also contained higher concentrations of thiacloprid compared to Stirling  
250 ( $\text{mean}_{\text{SU}} = 1.47\pm 4.41$  ng/g  $\text{median} < 0.03$  ng/g vs.  $\text{mean}_{\text{ST}} = 0.07\pm 0.22$  ng/g,  $\text{median} < 0.03$  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 ( $\text{mean}_{\text{ST}} = 0.16\pm 0.58$  g/g,  $\text{median} < \text{MDL}$ ,  
254  $\text{max}_{\text{ST}} = 2.08$  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  $p=0.560$ ;  $N_{\text{SU}}=13$ ,  $N_{\text{H}}=10$ ; Pollen  $p=0.142$ ,  $N_{\text{SU}}=13$ ,  $N_{\text{H}}=11$ ).  
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 ( $N= 85$ ,  $\text{mean}\pm \text{SD} = 0.64 \pm$   
266  $0.84$  ng/g,  $\text{median}=0.20$  ng/g,  $\text{max}= 4.23$  ng/g) were approximately three times the concentrations  
267 detected in pollen ( $N= 90$ ,  $\text{mean}\pm \text{SD} = 0.20 \pm 0.32$  ng/g,  $\text{median}\leq 0.12$  ng/g,  $\text{max}=1.74$  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%,  $\text{mean}_H \pm \text{SD}$   
278  $=1.46 \pm 0.66$  ng/g; median=1.17 ng/g; SU=93%,  $\text{mean}_{SU}=0.95 \pm 1.13$  ng/g, median  $\leq 0.12$  ng/g).  
279 Likewise, almost all pollen samples contained neonicotinoid residues (H=80%,  $\text{mean}_H=0.41 \pm 0.47$   
280 ng/g, median  $\leq 0.12$  ng/g; SU=100%,  $\text{mean}_{SU}=0.23 \pm 0.19$  ng/g, median  $\leq 0.12$  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,  $Z_{30}=0.28$ ,  $p=0.120$ ,  $\text{mean}_{\text{April}}=0.32 \pm 0.37$  ng/g, median  
285  $\leq 0.12$  ng/g  $\text{mean}_{\text{May}}=0.22 \pm 0.33$ , median  $\leq 0.12$  ng/g), neonicotinoid concentrations in nectar,  
286 previously high in comparison to pollen, declined significantly between April and May ( $Z_{26}=0.75$ ,  
287  $p<0.001$ ;  $\text{mean}_{\text{April}}=1.20 \pm 0.95$  ng/g, median= 1.06 ng/g,  $\text{mean}_{\text{May}}=0.65 \pm 0.72$ , 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  $\text{mean}_H=0.23 \pm 0.36$ , median  $\leq 0.12$  ng/g,  $\text{mean}_{SU}=0.17 \pm 0.27$ , median  $\leq 0.12$  ng/g), concentrations in

nectar were significantly higher in Hertfordshire hives ( $U_{44, 42}=1301$ ,  $p\leq 0.001$ ,  $\text{mean}_H=0.88\pm 0.81$ ,  
median=0.75 ng/g,  $\text{mean}_{SU}=0.40\pm 0.80$  ng/g, median  $\leq 0.10$  ng/g). Crop mapping of the five 5 km<sup>2</sup>  
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).

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

305

## 306 DISCUSSION

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

Across all samples, the highest neonicotinoid residue concentrations were detected in 2013, in  
pollen samples collected from rural bumblebee colonies in Sussex. Concentrations of up to 38.77 ng/g  
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<sup>25,15,26</sup>, and within the  
323 range demonstrated to have negative impacts on bumblebee physiology<sup>27,28</sup>, foraging efficiency<sup>29</sup> and  
324 colony growth<sup>28</sup>. 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<sup>22</sup>), 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<sup>14,15,23,30</sup>, and hypothesised to arise from temperature  
337 increases and photo-degradation of neonicotinoid residues in plant tissues as the season progresses<sup>31</sup>.  
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.*<sup>32</sup> (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<sup>32</sup>), 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<sup>33,34</sup>, and levels detected in pollen were within the range known to impair  
345 honeybee foraging performance<sup>35</sup>, immune function<sup>7</sup> and alter gene expression pathways<sup>36</sup>.  
346 Furthermore, as observed in several previous studies<sup>15,17,18</sup>, 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.*<sup>17</sup> 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<sup>37</sup>.

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.*<sup>30</sup> 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<sup>38</sup>.

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<sup>24</sup>.

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.<sup>30</sup> also noted that imidacloprid was present in honey samples  
386 harvested in 2014 at a rate ‘disproportional to its use’ and Tosi et al.<sup>17</sup> 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<sup>15</sup> and wild  
390 bumblebees collected in peri-urban areas<sup>23</sup>, 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<sup>24</sup>. 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<sup>15</sup>. 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<sup>39,40</sup>,  
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<sup>41</sup>, 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<sup>10-12</sup>. 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.

419

420

421

422

423

424

425

426

427

428

429

430

431

432

433 **FIGURES**

434

435

436

437

438

439

440

441

442

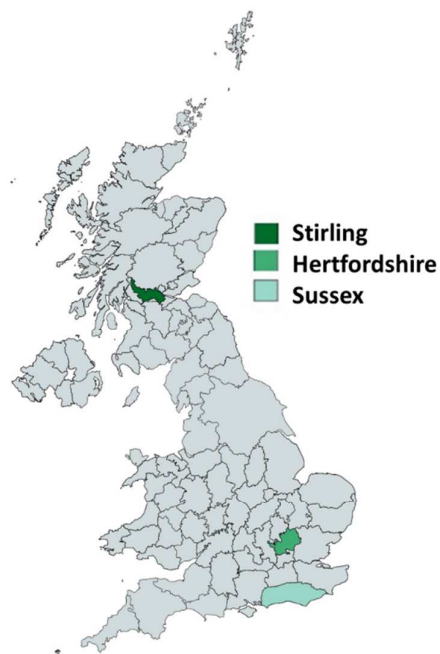
443

444

445

446

447



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).

452

453

454

455

456  
457  
458  
459  
460  
461

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

462  
463  
464  
465  
466  
467

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.



489	NECTAR										POLLEN									
	Method Quantification Limit (ng/g)										0.3	0.3	0.4	0.08	0.08	0.36	0.36	0.48	0.12	0.12
490	Method Detection Limit (ng/g)										0.1	0.1	0.14	0.03	0.03	0.12	0.12	0.16	0.04	0.04
491	Month	Region	N	TMX	CLO	IMC	ACT	THC	TOTAL	% Multi-residue	N	TMX	CLO	IMC	ACT	THC	TOTAL	% Multi-residue		
491	APRIL	HERTS	15	Frequency of detection %	100%	73.3%	6.7%			100%	80.0%	80%		6.6%		13.3%	80%	20.0%		
492				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	
493				Median (ng/g)	0.77	0.66	≤0.14				1.17			0.12		≤0.16		≤0.04	0.12	
494		SUSSEX	15	Max (ng/g)	1.83	1.38	≤0.14			1.83			0.94		≤0.16		1.62	1.62		
495				Frequency of detection %	93%	47%	7%		7%	93.3%	60.0%		100%						100%	0%
496				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
497	MAY	HERTS	15	Median (ng/g)	0	≤0.1	≤0.14	≤0.03	0.58			0.12		≤0.16		≤0.04	0.12			
498				Max (ng/g)	1.76	2.47	≤0.03	≤0.03	2.47				0.6						0.60	
499				Frequency of detection %	86.6%	73.3%				93.3%	66.7%		80%							80%
500		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		
501		Median (ng/g)	0.45	0.10				1.08			0.12							0.12		
502		Max (ng/g)	2.29	1.26				2.29			0.92							0.92		
503	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%			
504			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				
505			Median (ng/g)	0.10	≤0.10		≤0.03	0.10				≤0.12	≤0.12	≤0.16		≤0.04	0.1			
506	JUNE	HERTS	14	Max (ng/g)	0.53	0.68		≤0.03	0.68			≤0.12	≤0.12	≤0.16		1.19	1.2			
507				Frequency of detection %	50%	21.4%	7.1%			66.3%	21.4%		26.7%		6.7%				26.7%	8.9%
508				Mean ± SD (ng/g)	≤0.10	≤0.10	≤0.14			0.08±0.08		15	≤0.12		≤0.16				0.09±0.26	
509		Median (ng/g)	≤0.10	≤0.10	≤0.14			0.10			≤0.12		≤0.16				≤0.12			
510		Max (ng/g)	≤0.10	≤0.10	≤0.14			≤0.14			≤0.12		0.88				0.88			
511		SUSSEX	15	Frequency of detection %	13.3%				13.3%	0%		26.7%		6.7%		6.7%	33.3%	6.7%		
512	Mean ± SD (ng/g)			≤0.10					≤0.10		15	≤0.12		≤0.16		≤0.04	0.05±0.07			
513	Median (ng/g)			≤0.10					≤0.10			≤0.12		≤0.16		≤0.04	≤0.12			
514	Max (ng/g)	≤0.10					≤0.10			≤0.12		≤0.16		≤0.04	≤0.16					

505

506 Table 3 Frequency of detection (% samples), mean (± standard deviation (SD)), median and maximum concentrations of five neonicotinoids  
507 (TMX=thiamethoxam, CLO= clothianidin, IMC= imidacloprid, ACT=acetamiprid, THC=thiacloprid) and the combined total concentration of neonicotinoids  
508 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-  
509 residue samples are those where more than one type of neonicotinoid was detected. *MQL*= Method quantification limit, *MDL*=Method detection limit, *nt*= not  
510 tested, ≤ less than or equal to.

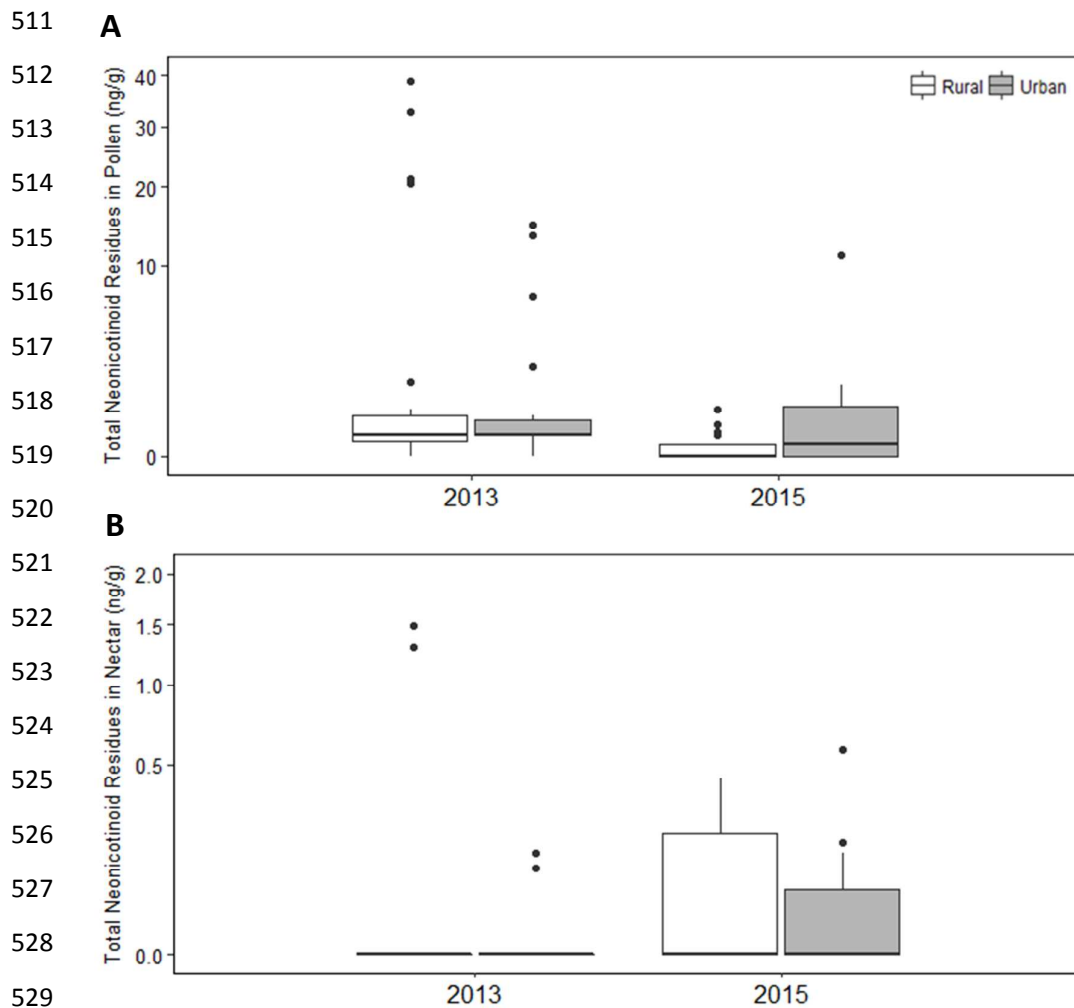


Figure 2 Total neonicotinoid concentrations (Thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid combined) detected in A) Pollen and B) Nectar samples collected from bumblebee colonies in Rural (White, N Pollen samples=45; Nectar=33) and Peri-urban (Grey, N Pollen samples=30; Nectar=25) 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 quartiles, and boxplot whiskers extend to 1.5 times the interquartile range. Outliers are represented by solid black circles.

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)

545 **AUTHOR INFORMATION**

546 **Corresponding Author**

547 \*Email: [e.nicholls@sussex.ac.uk](mailto:e.nicholls@sussex.ac.uk). Phone: +44(0)1273 873310

548 **Present Addresses**

549 <sup>¥</sup>Doñana Biological Station (EBD-CSIC), Integrative Ecology Department, Seville, ES-41092, Spain.

550

551 <sup>†</sup>Karlsruhe Institute of Technology (KIT), Institute of Meteorology and Climate Research–  
552 Atmospheric Environmental Research (IMK-IFU), 82467 Garmisch-Partenkirchen, Germany

553

554 <sup>§</sup> School of Geosciences, Grant Institute, King’s Buildings, Edinburgh, EH9 3JW, UK

555 <sup>‡</sup> French School of Public Health (EHESP), Research Institute for Environmental and Occupational

556 Health (Irset - Inserm UMR 1085), 35043 Rennes, France

557 **Notes**

558 The authors declare no competing financial interest.

559 **ACKNOWLEDGEMENTS**

560 We are grateful to BBSRC (BB/K014498/1; BB/J014915/1) and Defra (Research Project PS2372) for  
561 funding this work and for the farmers and land-owners who allowed us to work on their property and  
562 shared their pesticide usage data. We thank Bill Hughes, Mark Crumpton, Steve Kennedy and  
563 Luciano Scandian for beekeeping equipment, assistance and advice, and Pete Kennedy and Martyn  
564 Stenning for useful discussions regarding experimental design and logistics. Thanks also to Julia



565 Horwood and Daniel Ingram for technical advice and assistance and to Grace Twiston-Davies for  
566 digitising crop maps.

567 **REFERENCES**

- 568 (1) Simon-Delso, N.; Amaral-Rogers, V.; Belzunces, L. P.; Bonmatin, J. M.; Chagnon,  
569 M.; Downs, C.; Furlan, L.; Gibbons, D. W.; Giorio, C.; Girolami, V.; Goulson, D.  
570 Kreutzweiser, D. P.; Krupke, C.; H. Liess, M.; Long, E.; McField, M.; Mineau, P.;  
571 Mitchell, E.; A. D. Morrissey, C. A.; Noome, D. A.; Pisa, L.; Settele, J.; Stark, J. D.;  
572 Tapparo, A.; Van Dyck, H.; Van Praagh, J.; Van der Sluijs, J. P.; Whitehorn, P. R.;  
573 Wiemers, M. Systemic Insecticides (Neonicotinoids and Fipronil): Trends, Uses, Mode  
574 of Action and Metabolites. *Environ. Sci. Pollut. Res.* **2014**.
- 575 (2) Ollerton, J.; Winfree, R.; Tarrant, S. How Many Flowering Plants Are Pollinated by  
576 Animals? *Oikos* **2011**, *120* (3), 321–326.
- 577 (3) Alkassab, A. T.; Kirchner, W. H. Sublethal Exposure to Neonicotinoids and Related  
578 Side Effects on Insect Pollinators: Honeybees, Bumblebees, and Solitary Bees. *J. Plant*  
579 *Dis. Prot.* **2017**, *124* (1), 1–30.
- 580 (4) Whitehorn, P. R.; O'Connor, S.; Wackers, F. L.; Goulson, D. Neonicotinoid Pesticide  
581 Reduces Bumble Bee Colony Growth and Queen Production. *Science* **2012**, *336*  
582 (6079), 351–352.
- 583 (5) Gill, R. J.; Ramos-Rodriguez, O.; Raine, N. E. Combined Pesticide Exposure Severely  
584 Affects Individual- and Colony-Level Traits in Bees. *Nature* **2012**, *491* (7422), 105–  
585 108.
- 586 (6) Henry, M.; Béguin, M.; Requier, F.; Rollin, O.; Odoux, J.-F.; Aupinel, P.; Aptel, J.;  
587 Tchamitchian, S.; Decourtye, A. A Common Pesticide Decreases Foraging Success  
588 and Survival in Honey Bees. *Science* **2012**, *336* (6079), 348–350.
- 589 (7) Di Prisco, G.; Cavaliere, V.; Annoscia, D.; Varricchio, P.; Caprio, E.; Nazzi, F.;

- 590 Gargiulo, G.; Pennacchio, F. Neonicotinoid Clothianidin Adversely Affects Insect  
591 Immunity and Promotes Replication of a Viral Pathogen in Honey Bees. *Proc. Natl.*  
592 *Acad. Sci. U. S. A.* **2013**, *110* (46), 18466–18471.
- 593 (8) Laycock, I.; Cresswell, J. E. Repression and Recuperation of Brood Production in  
594 *Bombus Terrestris* Bumble Bees Exposed to a Pulse of the Neonicotinoid Pesticide  
595 Imidacloprid. *PLoS One* **2013**, *8* (11), e79872–e79872.
- 596 (9) European Commission. Commission Implementing Regulation (EU) No 485/2013 of  
597 24 May 2013 Amending Implementing Regulation (EU) No 540/2011, as Regards the  
598 Condition of Approval of the Active Substances Clothianidin, Thiamethoxam and  
599 Imidacloprid, and Prohibiting the Use and Sa. *Off. J. Eur. Union* **2013**, *L139/12*.
- 600 (10) European Commission. Commission Implementing Regulation (EU) 2018/783 of 29  
601 May 2018 Amending Implementing Regulation (EU) No 540/2011 as Regards the  
602 Conditions of Approval of the Active Substance Imidacloprid. *Off. J. Eur. Union* **2018**,  
603 *L 132/31*.
- 604 (11) European Commission. Commission Implementing Regulation (EU) 2018/784 of 29  
605 May 2018 Amending Implementing Regulation (EU) No 540/2011 as Regards the  
606 Conditions of Approval of the Active Substance Clothianidin. *Off. J. Eur. Union* **2018**,  
607 *L132/35*.
- 608 (12) European Commission. Commission Implementing Regulation (EU) 2018/785 of 29  
609 May 2018 Amending Implementing Regulation (EU) No 540/2011 as Regards the  
610 Conditions of Approval of the Active Substance Thiamethoxam. *Off. J. Eur. Union*  
611 **2018**, *L 132/40*.
- 612 (13) Carreck, N. L.; Ratnieks, F. L. W. The Dose Makes the Poison: Have “field Realistic”  
613 Rates of Exposure of Bees to Neonicotinoid Insecticides Been Overestimated in  
614 Laboratory Studies? *J. Apic. Res.* **2014**, *53* (5), 607–614.

- 615 (14) Botías, C.; David, A.; Horwood, J.; Abdul-Sada, A.; Nicholls, E.; Hill, E.; Goulson, D.  
616 Neonicotinoid Residues in Wildflowers, a Potential Route of Chronic Exposure for  
617 Bees. *Environ. Sci. Technol.* **2015**, *49* (21), 12731–12740.
- 618 (15) David, A.; Botías, C.; Abdul-Sada, A.; Nicholls, E.; Rotheray, E. L.; Hill, E. M.;  
619 Goulson, D. Widespread Contamination of Wildflower and Bee-Collected Pollen with  
620 Complex Mixtures of Neonicotinoids and Fungicides Commonly Applied to Crops.  
621 *Environ. Int.* **2016**, *88*, 169–178.
- 622 (16) Rundlöf, M.; Andersson, G. K. S.; Bommarco, R.; Fries, I.; Hederström, V.;  
623 Herbertsson, L.; Jonsson, O.; Klatt, B. K.; Pedersen, T. R.; Yourstone, J.; Smith, Henrik  
624 G. Seed Coating with a Neonicotinoid Insecticide Negatively Affects Wild Bees.  
625 *Nature* **2015**, *521*, 77–80.
- 626 (17) Tosi, S.; Costa, C.; Vesco, U.; Quaglia, G.; Guido, G. A 3-Year Survey of Italian  
627 Honey Bee-Collected Pollen Reveals Widespread Contamination by Agricultural  
628 Pesticides. *Sci. Total Environ.* **2018**, *615*, 208–218.
- 629 (18) Colwell, M. J.; Williams, G. R.; Evans, R. C.; Shutler, D. Honey Bee-Collected Pollen  
630 in Agro-Ecosystems Reveals Diet Diversity, Diet Quality, and Pesticide Exposure.  
631 *Ecol. Evol.* **2017**, *7* (18), 7243–7253.
- 632 (19) Balfour, N. J.; Al Toufailia, H.; Scandian, L.; Blanchard, H. E.; Jesse, M. P.; Carreck,  
633 N. L.; Ratnieks, F. L. W. Landscape Scale Study of the Net Effect of Proximity to a  
634 Neonicotinoid-Treated Crop on Bee Colony Health. *Environ. Sci. Technol.* **2017**, *51*  
635 (18), 10825–10833.
- 636 (20) Rolke, D.; Persigehl, M.; Peters, B.; Sterk, G.; Blenau, W. Large-Scale Monitoring of  
637 Effects of Clothianidin-Dressed Oilseed Rape Seeds on Pollinating Insects in Northern  
638 Germany: Residues of Clothianidin in Pollen, Nectar and Honey. *Ecotoxicology* **2016**,  
639 *25* (9), 1691–1701.

- 640 (21) Woodcock, B. A.; Bullock, J. M.; Shore, R. F.; Heard, M. S.; Pereira, M. G.; Redhead,  
641 J.; Ridding, L.; Dean, H.; Sleep, D.; Henrys, P.; Peyton, J.  
642 Hulmes, S.; Hulmes, L.; Sárospataki, M.; Saure, C.; Edwards, M.; Genersch, E.;  
643 Knäbe, S.; Pywell, R. F. Country-Specific Effects of Neonicotinoid Pesticides on  
644 Honey Bees and Wild Bees. *Science* (80-. ). **2017**, *356* (6345), 1393–1395.
- 645 (22) FERA PUS STATS for insecticides.
- 646 (23) Botías, C.; David, A.; Hill, E. M.; Goulson, D. Quantifying Exposure of Wild  
647 Bumblebees to Mixtures of Agrochemicals in Agricultural and Urban Landscapes.  
648 *Environ. Pollut.* **2017**, *222*, 73–82.
- 649 (24) Lentola, A.; David, A.; Abdul-Sada, A.; Tapparo, A.; Goulson, D.; Hill, E. M.  
650 Ornamental Plants on Sale to the Public Are a Significant Source of Pesticide Residues  
651 with Implications for the Health of Pollinating Insects. *Environ. Pollut.* **2017**, *228*,  
652 297–304.
- 653 (25) Scheper, J.; Holzschuh, A.; Kuussaari, M.; Potts, S. G.; Rundlöf, M.; Smith, H. G.;  
654 Kleijn, D. Environmental Factors Driving the Effectiveness of European Agri-  
655 Environmental Measures in Mitigating Pollinator Loss--a Meta-Analysis. *Ecol. Lett.*  
656 **2013**, *16* (7), 912–920.
- 657 (26) Botías, C.; David, A.; Hill, E. M.; Goulson, D. Contamination of Wild Plants near  
658 Neonicotinoid Seed-Treated Crops, and Implications for Non-Target Insects. *Sci. Total*  
659 *Environ.* **2016**, *566-567*, 269–278.
- 660 (27) Moffat, C.; Pacheco, J. G.; Sharp, S.; Samson, A. J.; Bolland, K. A.; Huang, J.;  
661 Buckland, S. T.; Connolly, C. N. Chronic Exposure to Neonicotinoids Increases  
662 Neuronal Vulnerability to Mitochondrial Dysfunction in the Bumblebee (*Bombus*  
663 *Terrestris*). *FASEB J.* **2015**, *29* (5), 2112–2119.
- 664 (28) Moffat, C.; Buckland, S. T.; Samson, A. J.; McArthur, R.; Chamosa Pino, V.; Bolland,

- 665 K. A.; Huang, J. T. J.; Connolly, C. N. Neonicotinoids Target Distinct Nicotinic  
666 Acetylcholine Receptors and Neurons, Leading to Differential Risks to Bumblebees.  
667 *Sci. Rep.* **2016**, *6*.
- 668 (29) Feltham, H.; Park, K.; Goulson, D. Field Realistic Doses of Pesticide Imidacloprid  
669 Reduce Bumblebee Pollen Foraging Efficiency. *Ecotoxicology* **2014**, *23* (3), 317–323.
- 670 (30) Woodcock, B. A.; Ridding, L.; Freeman, S. N.; Gloria Pereira, M.; Sleep, D.; Redhead,  
671 J.; Aston, D.; Carreck, N. L.; Shore, R. F.; Bullock, J. M.; Heard, Matthew S.;  
672 Pywell, Richard F. Neonicotinoid Residues in UK Honey despite European Union  
673 Moratorium. *PLoS One* **2018**, *13* (1).
- 674 (31) Bonmatin, J.-M.; Giorio, C.; Girolami, V.; Goulson, D.; Kreutzweiser, D. P.; Krupke,  
675 C.; Liess, M.; Long, E.; Marzaro, M.; Mitchell, E. a. D.; Noome, D. A.; Simon-Delso,  
676 N.; Tapparo, A. Environmental Fate and Exposure; Neonicotinoids and Fipronil.  
677 *Environ. Sci. Pollut. Res.* **2014**.
- 678 (32) Godfray, H. C. J.; Blacquièrre, T.; Field, L. M.; Hails, R. S.; Petrokofsky, G.; Potts, S.  
679 G.; Raine, N. E.; Vanbergen, A. J.; McLean, A. R. A Restatement of the Natural  
680 Science Evidence Base Concerning Neonicotinoid Insecticides and Insect Pollinators.  
681 *Proc. Biol. Sci.* **2014**, *281* (1786).
- 682 (33) Seeley, T. D.; Visscher, P. K. Survival of Honeybees in Cold Climates: The Critical  
683 Timing of Colony Growth and Reproduction. *Ecol. Entomol.* **1985**, *10* (1), 81–88.
- 684 (34) Wu-Smart, J.; Spivak, M. Sub-Lethal Effects of Dietary Neonicotinoid Insecticide  
685 Exposure on Honey Bee Queen Fecundity and Colony Development. *Sci. Rep.* **2016**, *6*.
- 686 (35) Cresswell, J. E. A Meta-Analysis of Experiments Testing the Effects of a  
687 Neonicotinoid Insecticide (Imidacloprid) on Honey Bees. *Ecotoxicology* **2011**, *20* (1),  
688 149–157.
- 689 (36) Christen, V.; Mittner, F.; Fent, K. Molecular Effects of Neonicotinoids in Honey Bees

- 690 (Apis Mellifera). *Environ. Sci. Technol.* **2016**, acs.est.6b00678.
- 691 (37) Iwasa, T.; Motoyama, N.; Ambrose, J. T.; Roe, R. M. M. Mechanism for the  
692 Differential Toxicity of Neonicotinoid Insecticides in the Honey Bee, *Apis Mellifera*.  
693 *Crop Prot.* **2004**, *23* (5), 371–378.
- 694 (38) Mitchell, E. A. D.; Mulhauser, B.; Mullet, M.; Mutabazi, A.; Glauser, G.; Aebi, A. A  
695 Worldwide Survey of Neonicotinoids in Honey. *Science (80)*. **2017**, *358* (6359), 109–  
696 111.
- 697 (39) Leonhardt, S. D.; Blüthgen, N. The Same, but Different: Pollen Foraging in Honeybee  
698 and Bumblebee Colonies. *Apidologie* **2011**, *43* (4), 1–16.
- 699 (40) Wood, T. J.; Holland, J. M.; Goulson, D. Pollinator-Friendly Management Does Not  
700 Increase the Diversity of Farmland Bees and Wasps. *Biol. Conserv.* **2015**, *187*, 120–  
701 126.
- 702 (41) EFSA. Evaluation of the Data on Clothianidin, Imidacloprid and Thiamethoxam for  
703 the 402 Updated Risk Assessment to Bees for Seed Treatments and Granules in the  
704 EU. *EFSA Support. Publ.* **2018**, *15*, 1–31.

705

706

707

708

709

710