

NEONICOTINOID RESIDUES IN WILDFLOWERS, A POTENTIAL ROUTE OF CHRONIC EXPOSURE FOR BEES

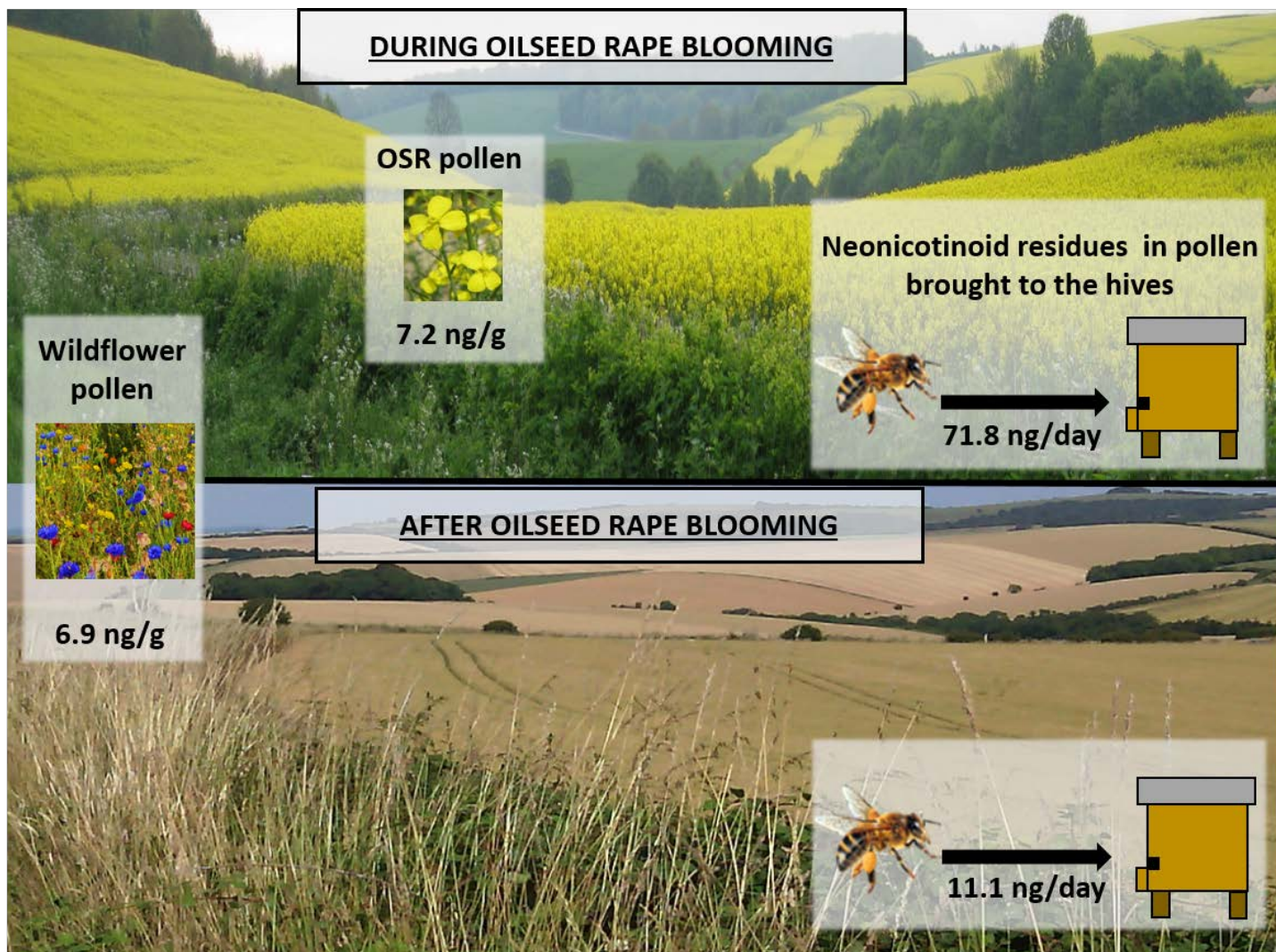
CRISTINA BOTÍAS, Arthur David, Julia Horwood, Alaa Abdul-
Sada, Elizabeth Nicholls, Elizabeth M Hill, and Dave Goulson

Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/acs.est.5b03459 • Publication Date (Web): 06 Oct 2015

Downloaded from <http://pubs.acs.org> on October 9, 2015

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 free 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 accessible to all readers and 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.



1 **NEONICOTINOID RESIDUES IN WILDFLOWERS, A POTENTIAL ROUTE OF**
2 **CHRONIC EXPOSURE FOR BEES**

3 Cristina Botías^{1*}, Arthur David¹, Julia Horwood¹, Alaa Abdul-Sada¹, Elizabeth Nicholls¹,
4 Elizabeth Hill¹, Dave Goulson¹

5 ¹School of Life Sciences, Sussex University, Falmer BN1 9QG, UK.

6 *Correspondence to: C.Botias@sussex.ac.uk. Phone: +44(0)1273872757

7

8

9 **TOC/Abstract art**

10

11

12

13

14

15

16

17

18

19

20

21

22

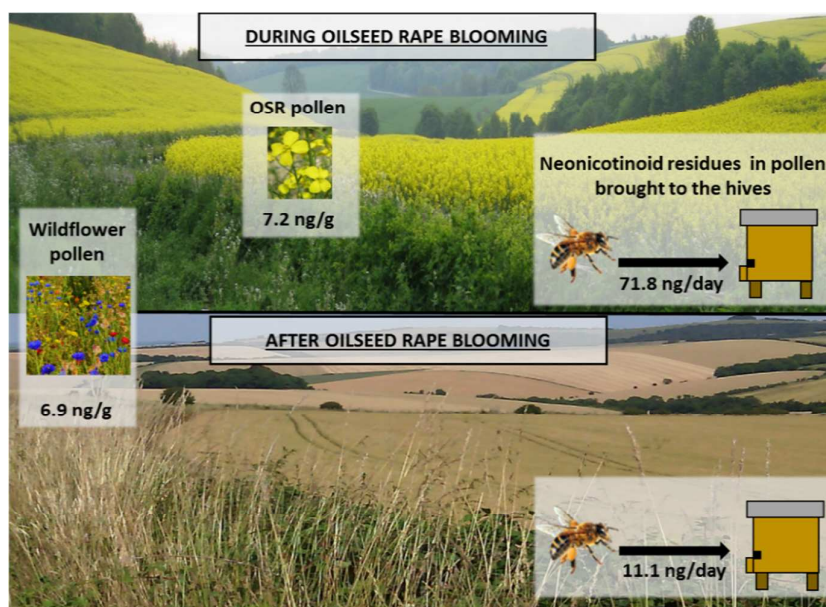
23

24

25 **Keywords:** Neonicotinoids; Pollinators; Oilseed Rape; Wildflowers; Environmental
26 Contamination

27

28



29 Abstract

30 In recent years, an intense debate has been generated about the environmental risks posed by
31 neonicotinoids, a group of widely-used, neurotoxic insecticides. When these systemic
32 compounds are applied to seeds, low concentrations are subsequently found in the nectar and
33 pollen of the crop, which are then **collected and** consumed by bees. Here we demonstrate that
34 current focus on exposure to pesticides via the crop overlooks an important factor –
35 throughout spring and summer, mixtures of neonicotinoids are also found in the pollen and
36 nectar of wildflowers growing in arable field margins, at concentrations that are sometimes
37 even higher than those found in the crop. Indeed the large majority (97%) of neonicotinoids
38 brought back in pollen to honey bee hives in arable landscapes was from wildflowers, not
39 crops. Both previous and ongoing field studies have been based on the premise that exposure
40 to neonicotinoids would only occur during the blooming period of flowering crops and that it
41 may be diluted by bees also foraging on untreated wildflowers. Here, we show that exposure
42 is likely to be higher and more prolonged than currently recognized due to widespread
43 contamination of wild plants growing near treated crops.

44 Introduction

45 Bees currently face many interacting pressures including loss of habitat and concomitant
46 reductions in the availability of flowers and nest sites, impacts of parasites and pathogens
47 (both native and introduced), and exposure to pesticides.¹ **The contribution of pesticides, and**
48 **in particular neonicotinoids, to pollinator declines has led to controversy across the United**
49 **States and Europe.**² Laboratory and semi-field studies on honey bees and bumblebees suggest
50 that exposure of colonies to concentrations approximating those found in pollen and nectar of
51 flowering crops can impair pollen collection, increase worker mortality, weaken immune
52 function, reduce nest growth and the production of new queens.^{3–6} However, a key point of
53 controversy is whether bees consume enough of these compounds during the flowering
54 period of the crop to do them significant harm. It has thus been argued that the levels of
55 exposure used in these studies may be higher than most bee colonies are likely to experience
56 in the field, based on the premise that exposure to neonicotinoids from flowering crops will
57 be diluted by bees also foraging on untreated wildflowers.⁷ **Moreover, it has been shown that**
58 **the concentrations of neonicotinoid residues present in food stores are extremely variable,**
59 **going from no detectable levels to more than 200 ng/g in bee stored pollen.**^{8–10} Some field
60 studies where honey bee hives were exposed to plots of treated crops for the duration of their
61 flowering period found no measurable impact on colony health.^{11–14} A recent well-replicated
62 and realistic field study found that exposure to a treated oilseed rape crop for one season was
63 not enough to have **measurable adverse effects** on honey bee colonies, but did have profound
64 effects on bumblebee nests and on reproduction of solitary bees, suggesting that honey bees
65 may be more able to cope with exposure to neonicotinoids than wild bees.¹⁴

66 Here, we present data on environmental contamination with neonicotinoids from five
67 predominantly arable farms in East Sussex, UK. We sampled soil from fields under
68 neonicotinoid-treated winter oilseed rape (OSR) in spring 2013, and also soil from beneath
69 the herbaceous vegetation in the field margins of both OSR and winter wheat crops. We

70 sampled by hand the pollen and nectar of the OSR crop, and of the wildflowers growing in
71 the margins of both winter wheat and OSR fields through the spring and summer. We also
72 placed honey bee colonies on these farms and sampled the pollen returned to the hives, to
73 estimate the level of exposure to neonicotinoids. Finally, we analysed samples of
74 neonicotinoid-dressed seeds, and of crop seeds untreated with neonicotinoids for sowing
75 during the EU moratorium. The objectives of this study were to evaluate the environmental
76 contamination caused by the application of neonicotinoid seed treatments in conventional
77 arable farms and to examine the role of non-target vegetation as a source of exposure to
78 neonicotinoid residues for bees.

79 **Materials and Methods:**

80 1. SAMPLE COLLECTION METHODS

81 1.1. Sampling locations

82 Seven winter-sown oilseed rape (sown at the end of August 2012) and five winter-sown
83 wheat (WW, sown at the end of September 2012) fields were selected at random from five
84 conventional farms located in East Sussex, South-East England, UK. The selected fields had
85 varying cropping history following normal farming practices in the region (the predominant
86 crops being WW and OSR). Previous crops had been treated with a range of pesticides,
87 including use of neonicotinoids each year for at least the three previous years (SI Table S1a-
88 S1g). The seeds from the OSR fields were all treated with Cruiser® seed dressing in 2012
89 (active ingredients: 280 g/L thiamethoxam, 8 g/L fludioxonil and 32.2 g/L metalaxyl-M) and
90 the WW was treated with Redigo® Deter® (a.i.: 50 g/L prothioconazole and 250 g/L
91 clothianidin) following normal farming practice.

92 1.2. Analysis of commercial oilseed rape, wheat and barley seeds.

93 In order to determine relative concentrations of neonicotinoid insecticides in commercial
94 seeds routinely used in UK farmland we tested one sample of rape seeds treated at a
95 purported rate of 4.2 g a.i. thiamethoxam per kg seed (Cruiser OSR®), and one wheat sample
96 with 0.5 g a.i. clothianidin per kg seed (Redigo® Deter). Additionally, fungicide only treated
97 seeds were analysed, using oilseed rape seeds treated with Agrichem® HY-PRO Duet (a.i.
98 150 g/L prochloraz, 333 g/L thiram), oilseed rape seeds treated with Beret Multi® (a.i. 25 g/L
99 fludioxonil, 25 g/L flutriafol), and barley seeds treated with Kinto® (a.i. 20 g/L triticonazole,
100 60 g/L prochloraz).

101 1.3. Soil sampling.

102 Soil samples were collected from the 7 OSR fields ten months after sowing (June 2013).
103 Three sites of 50 m² were sampled in each field, sites being at least 100 m apart. Within each
104 site, 15 x 20 g subsamples were collected at 0 – 10 cm depth and pooled to minimise
105 variation due to small-scale heterogeneity in pesticide concentrations.

106 Soil from the margins was also sampled from all four margins of 5 of the OSR fields and 5
107 WW fields. As above, each sample comprised a pool of 15 subsamples collected along the
108 length of the margin at 0-10 cm depth. The average sample distance from the crop edge was

109 1.5 m (range 1-2 m). Only soil samples from the margins where neonicotinoid pesticides
110 were detected in wildflowers were analysed (24 of 120 samples) Field margin soil samples
111 were only analysed if neonicotinoids were detected in wildflowers in that margin, since our
112 goal was to examine whether soil was a plausible route for contamination of the flowers.-

113 All soil samples were stored on ice in coolers in the field and then frozen immediately in the
114 laboratory and kept at -80°C.

115 1.4. Pollen and nectar samples collected from oilseed rape plants.

116 Nectar and pollen samples were collected during the period of rape blooming (from the 19th
117 of May to 27th of June 2013) directly from rape flowers in the 7 OSR fields using the same
118 three sampling sites per field as for the soil samples. Additional details are provided in the
119 Supporting Information (SI).

120 1.5. Pollen and nectar samples collected from wild plants in the field margins.

121 Field boundaries in the region typically consist of a hedge of woody plants separated from the
122 crop by a 0-2 m strip of herbaceous vegetation. Samples of pollen and nectar were collected
123 from the wild flowers that were present in the field margins and hedge choosing
124 representatives of the main plant families of which honey bees and other bees feed, using the
125 same methodology as for OSR plants (see SI). A total of 57 nectar samples and 188 pollen
126 samples from 54 different plant species were gathered from the same field margins where the
127 soil samples were collected. The species of wildflowers collected varied considerably and
128 depended upon which species were available. The average sample distance from the crop
129 edge was 1.5 m (range 1-2 m). When the weight of pollen samples or the volume of nectar
130 samples were not high enough to be analysed separately, samples from different species
131 growing in the same or neighbouring margin were pooled and analysed as a single sample. In
132 total, 55 out of 98 of the wildflower pollen samples (56.1%), and 21 out of 32 of the
133 wildflower nectar samples (67.7%) could be analysed as single species, and the rest were all
134 analysed as pooled samples from different species (see SI Tables S2a-S2j and Tables S8a-
135 S8b).

136 1.6. Pollen collected by honey bees.

137 Five honey bee (*Apis mellifera*) colonies (1 hive per farm) were placed in the vicinity of OSR
138 fields at the beginning of the OSR flowering period (May 2013), and remained at the same
139 sites until the end of August 2013. The hives were equipped with pollen traps during 4
140 consecutive days at the beginning of June 2013, and for 4 days in mid-August 2013 in order
141 to collect pollen loads from the returning honey bee foragers during the OSR blooming
142 period, and also when no OSR was in flower. After 4 days, the traps were removed and the
143 honey bee collected pollen loads were stored on ice and then at -80 °C in the laboratory until
144 analysis. Pollen loads within each sample were sorted by eye according to colour, texture,
145 size and shape as indicators of different pollen types. All pollen types were separately
146 weighed to calculate their relative abundance within the samples.^{15,16} A representative sample
147 of loads from each pollen type was mounted and pollen grains were identified under a
148 microscope following standard methods¹⁷ and using reference specimens and published
149 reference collections.¹⁸⁻²¹

150 1.7. Residue analysis

151 - Sample preparation for neonicotinoid analyses

152 All samples were analysed for concentrations of thiamethoxam (TMX), clothianidin (CLO),
153 imidacloprid (IMC) and thiacloprid (THC). Additional details are provided in the Supporting
154 Information.

155 *Soil and seed samples*

156 One hundred grams of each soil sample was homogenised and sieved (2 mm), and 100 g of
157 seed samples were ground to a fine powder with a mortar and pestle. An aliquot of soil or
158 seed samples (0.5 g \pm 0.5 g for both matrices) was spiked with 1 ng of the deuterated
159 pesticides in ACN and extracted using the QuEChERS method. First, 2 ml of water was
160 added to form an emulsion and samples were then extracted by adding 2.5 ml of ACN and
161 750 μ l of hexane and mixing on a multi axis rotator for 10 minutes. Then, 1.25 g of
162 magnesium sulphate: sodium acetate mix (4:1) was added to each tube in turn with immediate
163 shaking to disperse the salt and prevent clumping of the magnesium salt. After centrifugation
164 (13,000 RCF for 5 min), the supernatant was removed into a clean Eppendorf tube containing
165 625 mg of SupelTMQuE PSA/C18/ENVI-Carb and vortexed. The aqueous phase and salt
166 pellet were extracted again using 1.75 ml ACN and the supernatant combined with the
167 previous ACN extract. The extract was mixed with PSA/C18/ENVI-Carb on a multi axis
168 rotator (10 min) and then centrifuged (10 min). The supernatant was transferred into a glass
169 tube, evaporated to dryness under vacuum, reconstituted with 200 μ l of ACN:H₂O (10:90)
170 and spin filtered (0.22 μ m). Seed samples were then further diluted in order to be able to
171 determine thiamethoxam and clothianidin concentrations. An aliquot of 1.5 g of each wet soil
172 sample was dried for 24 hours at 105°C to determine the water content, and neonicotinoid
173 concentrations were expressed as **ng/g** dry weight of soil.

174 *Pollen*

175 One hundred milligrams of pollen sample was weighed into an Eppendorf tube and 150 μ g of
176 deuterated pesticides in ACN were added and the samples were extracted using the
177 QuEChERS method. The same ratio of solvents, salts and PSA/C18/ENVI-Carb per g of
178 sample as for the soil extractions was used (*i.e.*, 400 μ l of water, 500 μ l of ACN, 150 μ l of
179 hexane, 250 of magnesium sulphate: sodium acetate mix (4:1) and 125 mg of
180 PSA/C18/ENVI-Carb). After the first extraction, the aqueous phase and resuspended pellet
181 were extracted again with 400 μ l of ACN and the supernatants combined. Extracts were
182 mixed with PSA/C18/ENVI-Carb (10 min) and centrifuged (10 min). The supernatant was
183 evaporated to dryness under vacuum, reconstituted with 120 μ l of ACN:H₂O (10:90) and
184 filtered as above.

185 *Nectar*

186 Nectar in the capillary tube was expelled into an eppendorf tube and the capillary was then
187 flushed in 100 μ l of H₂O:ACN (90:10) and combined with the nectar sample. The nectar
188 samples were centrifuged at 13,000 relative centrifugal force (RCF) for 10 min to remove
189 pollen and plant debris and the supernatant (between 10 and 110 μ l dependent on collection
190 volume) transferred into a clean eppendorf tube and made up to 200 μ l using H₂O:ACN

191 (90:10). Fifty pg of deuterated pesticide standard mixture was added to 200 μ l diluted nectar
192 and the samples were extracted using the first step of the QuEChERS method. For this, 250
193 μ l of ACN were added and samples were extracted on a multi axis rotator for 10 min. Then
194 125 mg of magnesium sulphate: sodium acetate mix (4:1) was added, shaken (3 min) and
195 centrifuged (13,000 RCF, 5 min). The supernatant was removed and the aqueous phase
196 extracted again with 250 μ l of ACN and the supernatants combined. Samples were
197 reconstituted in 50 μ l of H₂O:ACN (90:10), centrifuged (13,000 RCF, 10 min) prior to
198 UHPLC-MS/MS analysis.

199 *UHPLC-MS/MS analyses*

200 Ultra high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS)
201 analyses were carried out using a Waters Acquity UHPLC system coupled to a Quattro
202 Premier triple quadrupole mass spectrometer from Micromass (Waters, Manchester, UK).
203 Samples were separated using a reverse phase Acquity UHPLC BEH C18 column (1.7 μ m,
204 2.1 mm \times 100 mm, Waters, Manchester, UK) fitted with a ACQUITY UHPLC BEH C18
205 VanGuard pre-column (130 \AA , 1.7 μ m, 2.1 mm \times 5 mm, Waters, Manchester, UK)
206 maintained at 22 $^{\circ}$ C. Injection volume was 20 μ l and mobile phase solvents were 95% water,
207 5% ACN, 5 mM ammonium formate, 0.1% formic acid (A) and 95% ACN, 5% water, 5 mM
208 ammonium formate, 0.1% formic acid (B). Initial ratio (A:B) was 90:10 and separation was
209 achieved using a flow rate of 0.2 ml/min with the following gradient: 90:10 to 70:30 in 10
210 min; then from 70:30 to 0:100 in two minutes and held for 7 min, and return to initial
211 condition and equilibration for 7 min.

212 MS/MS was performed in Multiple Reaction Mode (MRM) using ESI in the positive mode
213 and two characteristic fragmentations of the deprotonated molecular ion $[M+H]^+$ were
214 monitored; the most abundant one for quantitation and the second one used as a qualifier.
215 Retention times, ionisation and fragmentation settings are reported as SI Table S5. Other
216 parameters were optimised as follows: capillary voltage -3.3 kV, extractor voltage 8 V,
217 multiplier voltage 650 V, source temperature 100 $^{\circ}$ C, desolvation temperature 300 $^{\circ}$ C. Argon
218 was used as collision gas (P collision cell: 3×10^{-3} mbar), while nitrogen was used as
219 desolvation gas (600 L/h). Mass calibration of the spectrometer was performed with sodium
220 iodide. Samples were analysed in a random order and QC samples (i.e. standards) were
221 injected during runs every 10 samples to check the sensitivity of the machine. Data were
222 acquired using MassLynx 4.1 and the quantification was carried out by calculating the
223 response factor of neonicotinoid compounds to their respective internal standards.
224 Concentrations were determined using a least-square linear regression analysis of the peak
225 area ratio versus the concentration ratio (native to deuterated). At least five point calibration
226 curves ($R^2 > 0.99$) were used to cover the range of concentrations observed in the different
227 matrices for all compounds, within the linear range of the instrument. Method detection and
228 quantification limits (MDL and MQL, respectively) were determined from spiked samples
229 which had been extracted using the QuEChERS method. Non-spiked samples were also
230 prepared. MDLs were determined as the minimum amount of analyte detected with a signal-
231 to-noise ratio of 3 and MQLs as the minimum amount of analyte detected with a signal-to-

232 noise ratio of 10, after accounting for any levels of analyte present in non-spiked samples (SI
233 Table S6a).

234 *Quality control*

235 One blank workup sample (*i.e.* solvent without matrix) per batch of eleven samples was
236 included and injected on the UHPLC-MS/MS to ensure that no contamination occurred
237 during the sample preparation. Solvent samples were also injected between sample batches to
238 ensure that there was no carryover in the UHPLC system that might affect adjacent results in
239 analytical runs. Several replicates per site were analysed and all samples in which pesticides
240 were detected were extracted and analysed at least in duplicate for confirmation. Identities of
241 detected neonicotinoids were confirmed by comparing ratio of MRM transitions in samples
242 and pure standards. The QuEChERS method is used routinely for neonicotinoid analyses (e.g.
243 24) and recovery experiments performed on spiked (1 ng/g dw, n=4), pollen (1.2 ng/g dw,
244 n=4) and soil samples (10 ng/g dw, n=4) gave absolute recovery values ranging from 85 ± 8
245 to $111 \pm 5\%$ for the four pesticides in agreement with other published studies^{22,23} (SI Table
246 S6b). The concentration of any pesticides detected in unspiked samples was also determined
247 and subtracted from the spiked concentration to estimate the true recovery of the test
248 chemical. Finally, gas chromatography tandem mass spectrometry was also used to confirm
249 the high thiamethoxam concentrations observed in some wildflower pollen samples (see
250 Supporting Information).

251 1.8. Statistical analysis

252 All statistical analyses were carried out using SPSS 21 software. To test for differences in the
253 concentrations of the neonicotinoids in soil from OSR fields and field margins a two-way
254 ANOVA procedure was used (OSR fields 1 to 5, where samples from both cropland and
255 margins were collected) with the origin of samples (cropland or field margins) as fixed
256 factors and the concentrations for the different neonicotinoids (TMX, CLO, IMC, THC and
257 total neonicotinoid residues) as response variables. When no statistically significant
258 interaction was found, this term was removed from the model and the analysis was rerun to
259 test for the main effects of the fixed factors, using Tukey post hoc test for multiple
260 comparisons.

261 One-way ANOVA procedure was used to test for possible differences in concentrations of
262 neonicotinoid residues among the 7 fields where OSR pollen samples were collected (OSR
263 fields 1-7), followed by Tukey or Tamhane post hoc tests for multiple comparisons
264 depending on the homogeneity of variance in each case (determined using Levene's test).
265 Levels in nectar were also compared among the 7 OSR fields using Kruskal-Wallis test (K-
266 W) due to non-normality in the distribution of the data.

267 Non-parametric Mann-Whitney U-tests (M-W) were used to compare the concentrations of
268 neonicotinoids present in pollen and nectar collected from OSR flowers; to compare pollen
269 and nectar collected from OSR flowers *vs.* pollen and nectar from wildflowers growing in the
270 OSR field margins; for pollen collected from wildflowers growing in OSR field margins *vs.*
271 wildflowers from WW field margins; for pollen collected from wildflowers growing in the
272 OSR and WW margins *vs.* honey bee collected pollen of wildflower origin; and for pollen

273 collected by the honey bees in June vs. collected in August. To perform the statistical
274 analyses, all concentrations that were over the limits of detection (\geq MDL) but below the
275 limits of quantification ($<$ MQL) were assigned the value considered as the MDL in each case
276 (SI Table S65a). Concentrations below the MDL were considered to be zero.

277 Pearson's coefficient of correlation (for normally distributed data) and Spearman's rank
278 correlation (for data not normally distributed) were used to assess the relationship among
279 levels of neonicotinoids in nectar, pollen and soil from collected in the OSR fields. When the
280 relationship between levels in nectar and pollen or soil was evaluated, as the number of
281 samples for nectar was reduced from 21 to 13 due to small volumes for some samples, the
282 number of data for pollen ($N = 21$) and soil ($N = 21$) was reduced accordingly by calculating
283 means where necessary. The number of samples was not reduced when the relationship in the
284 levels of neonicotinoids was evaluated between pollen and soil.

285 The coefficient of variation (C_v) in the concentrations of neonicotinoids found in OSR pollen
286 and OSR nectar, and in wildflower pollen was used to analyse the consistency in the levels
287 found in these sets of samples, using t -tests to compare between the variability found in OSR
288 pollen vs. OSR nectar, and in OSR pollen vs. wildflower pollen.

289 The diversity of plant taxa represented in pollen collected by honey bees per site and
290 sampling period was calculated using Simpson's index of Diversity (1-D).²⁴

291

292 **Results and Discussion**

293 - Soil samples from OSR cropland and margins, and WW field margins.

294 All soil samples taken under OSR ($N = 21$) tested positive for thiamethoxam, which was the
295 dressing applied to the seeds of the current crop, and for clothianidin, a breakdown product of
296 thiamethoxam (Table 1). However, samples also all tested positive for imidacloprid and
297 42.9% tested positive for thiacloprid, though these two compounds had not been applied in
298 the previous three years (SI Tables S1a-S1g). The field margin soils adjacent to OSR ($N =$
299 16) also all contained thiamethoxam and clothianidin, but the concentrations of these two
300 compounds were significantly lower to the ones found in soil from OSR cropland (two-way
301 ANOVA: $F(1,25) = 12.78$, $P = 0.001$, $\eta_p^2 = 0.338$ (thiamethoxam); $F(1,25) = 14.51$, $P =$
302 0.001 , $\eta_p^2 = 0.367$ (clothianidin)). Imidacloprid was detected in all but one (93.8%) of the
303 OSR margins, and thiacloprid, with lower levels in margins than in cropland as well (two-
304 way ANOVA: $F(1,25) = 1.326$, $P = 0.260$, partial $\eta_p^2 = 0.05$ (imidacloprid); $F(1,25) = 7.18$, $P =$
305 0.013 , partial $\eta_p^2 = 0.223$), was present in 25 % of the samples. The insecticide applied as
306 seed dressing in the WW fields was also found in all the soil samples from the WW margins
307 (clothianidin; $N = 8$; Table 1) together with imidacloprid in 75% of the samples,
308 thiamethoxam in 50% and thiacloprid in 25% of them. This widespread prevalence both in
309 cropland and in field margins is to be expected given the high persistence of these
310 compounds in soils^{25,26} and their high potential for lateral movement and leaching.²⁷⁻²⁹ **The**
311 **persistence of neonicotinoids increases under cool conditions, and in soils with higher pH,**

312 organic matter and mineral clay content,²⁶ but as these features were not evaluated in our
313 samples, their role in the persistence and concentrations found cannot be elucidated.

314 - Pollen and nectar samples collected from OSR plants.

315 Thiamethoxam used in the seed dressing was present in all pollen samples (21/21) and a
316 majority of nectar samples (7/13) collected from the OSR crops, at concentrations similar to
317 those found in previous studies^{26,30} and with no differences in the values for both matrices
318 (mean ng/g \pm SD: 3.26 \pm 2.16 ng/g in pollen, 3.20 \pm 4.61 ng/g in nectar; M-W test: $U(32) =$
319 90, $P > 0.05$, $Z = -1.65$; Table 2). Maximum concentrations were 11.1 and 13.3 ng/g for
320 pollen and nectar, respectively. In addition to thiamethoxam, 90.5% of the pollen samples
321 contained clothianidin and 85.7% contained thiacloprid. Regarding OSR nectar, 53.9% of the
322 samples presented thiacloprid, with lower levels than in pollen (M-W test: $U(32) = 50.0$, $P =$
323 0.002, $Z = -3.09$), and 30.8% contained clothianidin. The concentrations of the neonicotinoids
324 detected in the different samples were similarly highly variable for pollen and nectar (C_{V_OSR}
325 pollen = 82.75 \pm 66.04%; C_{V_OSR} nectar = 118.45 \pm 81.14 % for nectar; t-test: $t(6) = -0.681$, $P =$
326 0.521), and didn't show differences among the 7 fields where they were collected (e.g. TMX
327 in pollen samples: ANOVA, $F(6) = 2.46$, $P = 0.078$; TMX in nectar samples: K-W, $H(6) =$
328 10.12, $P = 0.120$). Furthermore, the concentrations for thiamethoxam in pollen were
329 positively correlated with the concentrations in the soil samples collected from the same sites
330 (Pearson correlation coefficient: $r(19) = 0.52$, $P = 0.017$; SI Fig. S1), but the same correlation
331 was not found for nectar (Spearman's rank correlation: $\rho(11) = -0.12$, $P = 0.70$).

332 - Pollen and nectar samples from wild plants in the field margins.

333 Pollen collected by hand from wildflowers in OSR field margins frequently contained
334 thiamethoxam (58% of 43 samples), sometimes at high concentrations, as in the case of a
335 pollen sample from *Heracleum sphondylium* (86 ng/g) collected in margin M2 of OSR field
336 4, and one from *Papaver rhoeas* (64 ng/g) collected in margin M2 of OSR field 1 (SI Tables
337 S2a and S2d). However, neonicotinoid residues were not always detected in pollen samples
338 of the same species collected from different field margins (SI Tables S2a-S2j). The possible
339 heterogeneity in soil properties and environmental factors along the field margins (e.g.
340 organic matter content, microbial communities, humidity, degree of slope, sunlight exposure)
341 may have influenced the persistence of neonicotinoids and their sorption onto soil particles in
342 specific sites,²⁶ thus resulting in a differential exposure and uptake of these active ingredients
343 by field margin plants growing in different field locations.

344 Overall, the total concentration of neonicotinoids present in the pollen from wildflowers in
345 the OSR field margins were higher than in pollen from the treated OSR plants (M-W test:
346 $U(62) = 287.0$, $P = 0.018$, $Z = -2.37$; Fig. 1), though as might be expected when testing a
347 range of different plant species, levels were more variable in wildflower samples ($C_{V_wildflower}$
348 pollen = 350.35 \pm 189.31 %; C_{V_OSR} pollen = 82.75 \pm 66.04 %; t-test: $t(6) = -2.669$, $P = 0.037$).
349 The higher residue levels detected in wildflower pollen was mainly due to the significantly
350 greater concentrations of thiamethoxam when compared to OSR pollen (M-W test: $U(62) =$
351 302.0, $P = 0.03$, $Z = -2.165$). In contrast, clothianidin and thiacloprid were typically found at
352 lower concentrations than in the crop (M-W test: $U(62) = 61.0$, $P < 0.001$, $Z = -6.36$

353 (clothianidin); $U(62) = 70.0$, $P < 0.001$, $Z = -6.64$ (thiacloprid); Fig. 1). Imidacloprid, absent
354 in OSR pollen, was detected in 11.6% of the wildflower pollen samples.

355 Residues of thiamethoxam, imidacloprid and thiacloprid were detected in pollen collected
356 from wildflowers adjacent to winter wheat fields, but the levels were lower (total
357 neonicotinoid residues = 0.17 ± 1.01 ng/g) than in wildflowers growing in OSR field margins
358 (total neonicotinoid residues = 15.40 ± 25.45 ng/g; M-W test: $U(96) = 507.0$, $Z = -5.75$, $P <$
359 0.001). The seed-treatment in the winter wheat fields, clothianidin, was not detected in any of
360 the pollen or nectar samples gathered from wildflowers growing in the WW field margins
361 (Table 2) despite being present in the soil beneath this margin vegetation (Table 1).
362 Thiamethoxam is more soluble in water (4.1 g/L) than clothianidin (0.30-0.34 g/L),³¹ and
363 thus it may have better systemic properties, increasing the probability for the uptake of this
364 compound by plants in comparison with clothianidin.

365 Only 20.8% (5 out of 24 samples) of the nectar samples obtained from wildflowers adjacent
366 to OSR crops contained thiamethoxam, and the concentrations for this compound ($0.10 \pm$
367 0.37 ng/g; Table 2) were significantly lower than for OSR nectar (3.20 ± 4.61 ng/g; M-W
368 test: $U(35) = 94.5$, $P = 0.049$, $Z = -2.3$; SI Tables S8a-S8b). We also found clothianidin in
369 20.8% of the nectar samples and thiacloprid in 25%, the latter presenting lower levels (all
370 detected levels were below MQL) than in OSR nectar (0.24 ± 0.36 ng/g; M-W test: $U(35) =$
371 90.0 , $P = 0.036$, $Z = -2.47$). The number of nectar samples obtained from wildflowers
372 adjacent to WW was low ($N = 8$) and none of them contained neonicotinoids residues. The
373 lower prevalence of neonicotinoid residues in nectar samples in comparison with pollen both
374 in OSR flowers and in wildflowers growing in the field margins may be due to the shorter
375 half-life of these compounds in aqueous matrices due to higher hydrolysis, photolysis and
376 microbial degradation.³²

377 Given that field-margin soils were found to be consistently contaminated with all of the
378 commonly-used neonicotinoids, this is the mostly likely source of wildflower contamination.
379 Three previous studies have demonstrated neonicotinoid contamination of wild plants
380 growing in field margins or surrounding areas of seed-treated crops, but in these studies the
381 whole flower was analysed³³ or the information about the part of the plant analysed was not
382 provided,^{14,34} so the concentrations found in the nectar or pollen and subsequent exposure to
383 bees was not clear. Our study marks a significant step towards understanding the prevalence
384 and concentrations of neonicotinoid residues present in pollen and nectar from non-target
385 plants, which are essential foraging sources for bees.³⁵

386 - Pollen collected by honey bees.

387 Pollen traps were used to collect pollen brought back to honey bee hives placed on the five
388 farms, both during the OSR blooming period (beginning of June 2013), and later in the
389 summer (mid-August 2013). Identification of pollen types revealed that the majority of pollen
390 collected by honey bees in June was *Crataegus monogyna* (62.5%), with just 9.9% of pollen
391 coming from OSR (SI Tables. S3a-S3b). Previous studies have indicated that honey bees may
392 not use OSR flowers as a major source of pollen,³⁶ but their frequent presence as pollinator
393 visitors in OSR crops³⁷⁻³⁹ could indicate that they may forage in OSR flowers mainly to

394 collect nectar.⁴⁰ In August the pollen loads were more diverse (Simpson's index of Diversity:
395 1-D = 0.85) than in June (1-D = 0.54), comprising a range of wildflowers with *Epilobium*
396 *hirsutum* (23.1%) and *Rubus fruticosus* (13.5%) the most visited plants. Honey bee collected
397 wildflower pollen commonly contained thiamethoxam, clothianidin, imidacloprid and
398 thiacloprid, but mean concentrations of total neonicotinoid residues were generally lower
399 (mean \pm s.d.: 1.48 \pm 4.56 ng/g) compared to pollen collected by hand from field margin
400 wildflowers (6.85 \pm 18.40 ng/g; M-W test: $U(171) = 2635.0$, $P = 0.001$, $Z = -3.389$) or from
401 the crop (7.20 \pm 5.08 ng/g; $U(94) = 110.5$, $P < 0.001$, $Z = -6.037$; Fig. 2). This is to be
402 expected since bees will have been foraging over a large area, visiting patches of wildflowers
403 that were not adjacent to crops, resulting in a dilution effect. It is notable that a significant
404 drop in the concentrations of neonicotinoids detected in wildflower pollen was observed
405 between June (3.09 \pm 6.45 ng/g) and August (0.20 \pm 0.43 ng/g; M-W test: $U(78) = 339.0$, $P <$
406 0.001 , $Z = -4.358$), perhaps suggesting a reduction in plant tissue concentrations through
407 summer due to photolysis⁴¹ and increasing temperatures.²⁶

408 Of the total neonicotinoid residues present in the pollen collected by honey bees in June (287
409 ng in 514 g of pollen; 0.56 ng residues/g pollen), only 3% had its origin in the OSR pollen,
410 the remaining 97% coming from wildflowers. In August, all identified pollen taxa were wild
411 plants (SI Fig. S1), residue levels were lower than in June, but also the amount of pollen
412 collected was smaller (44.28 ng of residues in 224.84 g of pollen; 0.20 ng residues/g pollen).
413 If one considers these values in terms of the quantity of neonicotinoid residues entering hives
414 per day, honey bee foragers brought back an amount of 71.8 ng of residues per day in June,
415 and 11.1 ng per day in August. According to current understanding, these concentrations are
416 lower than those likely to cause significant harm to honey bee colonies in the short term,^{30,10}
417 as for instance the oral LD₅₀ values (dose required to kill 50% of a population of test animals
418 in 48 h) for thiamethoxam and clothianidin in honey bees are 5 ng/bee and 3.7 ng/bee
419 respectively.⁴² Considering the mean values for neonicotinoid content in corbicular pollen
420 collected during oilseed rape bloom in this study (0.56 ng/g), a honey bee would need to eat
421 around 10 g of pollen to obtain an LD₅₀ dose, which is unlikely since honey bees consume
422 less than 10 mg of pollen per day.^{43,44} However, it should be noted that these figures do not
423 include the residues brought back to the hive in nectar, and that a long-term chronic exposure
424 to field realistic sub-lethal levels of thiamethoxam (5.31 ng/g) and clothianidin (2.05 ng/g)
425 has been shown to cause an impact on honey bee colony performance and queen
426 supersedure.⁴⁵ It is also worth mentioning that the number of colonies we used to evaluate
427 levels and origin of exposure to neonicotinoids on honey bee colonies was limited, and since
428 the overall foraging pattern may differ among colonies placed on the same landscapes due to
429 varying factors^{46,47}, a different outcome cannot be discarded with another experimental
430 design. Likewise, exposure of other bee species in this landscape will depend on their
431 foraging range and floral preferences, and may be quite different.

432 - Commercial oilseed rape, wheat and barley seeds.

433 Analysis of thiamethoxam-dressed OSR seeds revealed contamination with clothianidin (a
434 breakdown product of thiamethoxam) but also imidacloprid and thiacloprid (SI Table S4).
435 Most surprisingly, samples of OSR, winter wheat and barley seeds that had not been treated

436 with neonicotinoids and had been dressed only with fungicides also contained residues of
437 various mixtures of neonicotinoids, albeit at much lower concentrations than found in dressed
438 seeds. This may result either from contamination via the machinery used to dress or to count
439 the seeds, as suggested in a previous study where a similar contamination was detected in
440 cotton seeds,³⁴ or perhaps residues remaining from treatments to the crop from which the
441 seeds were harvested. The role of these additional neonicotinoid residues present in coated
442 seeds as a potential source of environmental contamination warrants further research.

443

444 Previous field studies of the impacts of neonicotinoids on bee colonies have often suffered
445 from contamination of control colonies.^{12,48} Our study provides a potential explanation for
446 this widespread presence of residues in bee colony food stores; much of the exposure of free-
447 flying bees is likely to be via residues in wildflowers, which cannot readily be manipulated.
448 In these circumstances we would not expect any differences in the performance of colonies
449 placed next to experimental plots of treated versus untreated crops, unless the experiment is
450 performed in a landscape where minimal neonicotinoids have been used previously.¹⁴

451 Farmers are often encouraged to sow wildflower strips in arable field margins as a means of
452 boosting pollinator populations and to attract and conserve natural enemies of arthropod
453 pests.^{49,50} Our data suggest that such wildflowers are likely to be contaminated with
454 neonicotinoids; whether the benefits accrued from providing more food and suitable habitat
455 would exceed the cost via impacts of the pesticide is unclear. However, when possible, it
456 would seem best to promote the creation of wildflower patches that are not adjacent to treated
457 crops or on soil in which treated crops have previously been grown to avoid exposure to
458 neonicotinoid residues via this route.

459 Overall, our results demonstrate that the application of neonicotinoid seed dressings to
460 autumn-sown arable crops results in contamination of pollen and nectar of nearby
461 wildflowers throughout the following spring and summer, and that wildflowers were the
462 major route of exposure for bees in this study. It has been suggested that chronic intake of
463 neonicotinoid pesticides may lead to weakening and failure in bee colonies^{45,51}, but the
464 consequences of prolonged exposure to mixtures of these compounds in wildflower pollen
465 and nectar have not been examined by any field study conducted to date. Furthermore,
466 widespread contamination of wild plants and soil is also likely to lead to chronic exposure of
467 a broad range of non-target invertebrates in farmland.

468

469 **Acknowledgements:** We are grateful to Defra (Research Project PS2372) and BBSRC
470 (BB/K014498/1) for funding this work, and to the five farmers for allowing us to work on
471 their property and sharing their pesticide usage data (information available in SI Tables S1a-
472 S1g). We also thank Martyn Stenning, Bill Hughes, Luciano Scandian, Anna Gorenflo, Jo
473 Bunner, Alfonso Herrera Bachiller, Elinor Jax, Tom Wood, Ellen Rotheray, Kate Basley,
474 Lena Grinsted, Julia Jones, Daniel Ingram and Rob Fowler for technical support and valuable
475 comments.

476 **Supporting Information Available:** Additional details for materials and methods, and tables
477 and figures as noted in the text. This material is available free of charge via the Internet at
478 <http://pubs.acs.org>

479 **Conflict of interests:** The authors declare no competing financial interest.

480

481 **References:**

- 482 (1) Goulson, D.; Nicholls, E.; Botias, C.; Rotheray, E. L. Bee declines driven by combined stress
483 from parasites, pesticides, and lack of flowers. *Science* **2015**, *347* (6229), 1255957.
- 484 (2) Suryanarayanan, S. Pesticides and pollinators: a context-sensitive policy approach. *Curr. Opin.*
485 *Insect Sci.* **2015**, *10*, 149–155.
- 486 (3) Whitehorn, P. R.; O'Connor, S.; Wackers, F. L.; Goulson, D. Neonicotinoid Pesticide Reduces
487 Bumble Bee Colony Growth and Queen Production. *Science* **2012**, *336* (6079), 351–352.
- 488 (4) Gill, R. J.; Ramos-Rodriguez, O.; Raine, N. E. Combined pesticide exposure severely affects
489 individual- and colony-level traits in bees. *Nature* **2012**, *491* (7422), 105–108.
- 490 (5) Laycock, I.; Lenthall, K. M.; Barratt, A. T.; Cresswell, J. E. Effects of imidacloprid, a
491 neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*).
492 *Ecotoxicology* **2012**, *21* (7), 1937–1945.
- 493 (6) Di Prisco, G.; Cavaliere, V.; Annoscia, D.; Varricchio, P.; Caprio, E.; Nazzi, F.; Gargiulo, G.;
494 Pennacchio, F. Neonicotinoid clothianidin adversely affects insect immunity and promotes
495 replication of a viral pathogen in honey bees. *Proc. Natl. Acad. Sci.* **2013**, *110* (46), 18466–
496 18471.
- 497 (7) Godfray, H. C. J.; Blacquière, T.; Field, L. M.; Hails, R. S.; Petrokofsky, G.; Potts, S. G.;
498 Raine, N. E.; Vanbergen, A. J.; Mclean, A. R.; B, P. R. S.; et al. A restatement of the natural
499 science evidence base concerning neonicotinoid insecticides and insect pollinators. *Proc. R.*
500 *Soc. B* **2014**, *281*, 20140558.
- 501 (8) Bernal, J.; Garrido-Bailón, E.; Del Nozal, M. J.; González-Porto, A. V.; Martín-Hernández, R.;
502 Diego, J. C.; Jiménez, J. J.; Bernal, J. L.; Higes, M. Overview of Pesticide Residues in Stored
503 Pollen and Their Potential Effect on Bee Colony (*Apis mellifera*) Losses in Spain. *J. Econ.*
504 *Entomol.* **2010**, *103* (6), 1964–1971.
- 505 (9) Mullin, C. A.; Frazier, M.; Frazier, J. L.; Ashcraft, S.; Simonds, R.; VanEngelsdorp, D.; Pettis,
506 J. S. High levels of miticides and agrochemicals in North American apiaries: implications for
507 honey bee health. *PLoS One* **2010**, *5* (3), e9754.
- 508 (10) Blacquière, T.; Smagghe, G.; van Gestel, C. M.; Mommaerts, V. Neonicotinoids in bees: A
509 review on concentrations, side-effects and risk assessment. *Ecotoxicology* **2012**, *21* (4), 973–
510 992.
- 511 (11) Cutler, G. C.; Scott-Dupree, C. D. Exposure to clothianidin seed-treated canola has no long-
512 term impact on honey bees. *Ecotoxicology* **2007**, *100* (3), 765–772.

- 513 (12) Cutler, G. C.; Scott-Dupree, C. D.; Sultan, M.; McFarlane, A. D.; Brewer, L. A large-scale
514 field study examining effects of exposure to clothianidin seed-treated canola on honey bee
515 colony health, development, and overwintering success. *PeerJ* **2014**, *2*, e652.
- 516 (13) Pilling, E.; Campbell, P.; Coulson, M.; Ruddle, N.; Tornier, I. A four-year field program
517 investigating long-term effects of repeated exposure of honey bee colonies to flowering crops
518 treated with thiamethoxam. *PLoS One* **2013**, *8* (10), e77193.
- 519 (14) Rundlöf, M.; Anderson, G. K. S.; Bommarco, R.; Fries, I.; Hederstrom, V.; Herbertsoon, L.;
520 Jonsson, O.; Klatt, B. K.; Pedersen, T. R.; Yourstone, J.; et al. Seed coating with a
521 neonicotinoid insecticide negatively affects wild bees. *Nature* **2015**, *521*, 77–80.
- 522 (15) Kirk, W. D. J. *A Colour Guide to Pollen Loads of the Honey Bee*, 2nd Edition; International
523 Bee Research Association, **2006**.
- 524 (16) Human, H.; Brodschneider, R.; Dietemann, V.; Dively, G.; Ellis, J. D.; Forsgren, E.; Fries, I.;
525 Hatjina, F.; Hu, F.-L.; Jaffé, R.; et al. Miscellaneous standard methods for *Apis mellifera*
526 research. *J. Apic. Res.* **2013**, *52* (4), 1–56.
- 527 (17) Delaplane, K. S.; Dag, A.; Danka, R. G.; Freitas, B. M.; Garibaldi, L. a; Goodwin, R. M.;
528 Hormaza, J. I. Standard methods for pollination research with *Apis mellifera*. *J. Apic. Res.*
529 **2013**, *52* (4), 1–28.
- 530 (18) Sawyer, R. *Pollen Identification for Beekeepers*; Pickard, R. S., Ed.; University College
531 Cardiff Press: Cardiff, **1981**.
- 532 (19) Demske, D.; Tarasov, P. E.; Nakagawa, T. Atlas of pollen, spores and further non-pollen
533 palynomorphs recorded in the glacial-interglacial late Quaternary sediments of Lake Suigetsu,
534 central Japan. *Quat. Int.* **2013**, *290-291*, 164–238.
- 535 (20) Moore, P.D., Webb, J.A., Collinson, M. E. *Pollen Analysis*; Wiley-Blackwell, **1991**.
- 536 (21) Galarini, R., Ricciardelli D'Albore, M. Mediterranean Melissopalynology
537 <http://www.izsum.it/Melissopalynology/>.
- 538 (22) Kamel, A. Refined methodology for the determination of neonicotinoid pesticides and their
539 metabolites in honey bees and bee products by liquid chromatography-tandem mass
540 spectrometry (LC-MS/MS). *J. Agric. Food Chem.* **2010**, *58* (10), 5926–5931.
- 541 (23) Chen, M.; Collins, E. M.; Tao, L.; Lu, C. Simultaneous determination of residues in pollen and
542 high-fructose corn syrup from eight neonicotinoid insecticides by liquid chromatography-
543 tandem mass spectrometry. *Anal. Bioanal. Chem.* **2013**, *405*, 9251–9264.
- 544 (24) Peet, R. K. The measurement of species diversity. *Annu. Rev. Ecol. Evol. Syst.* **1974**, *5* (1),
545 285–307.
- 546 (25) Goulson, D. An overview of the environmental risks posed by neonicotinoid insecticides. *J.*
547 *Appl. Ecol.* **2013**, *50* (4), 977–987.
- 548 (26) Bonmatin, J.-M.; Giorio, C.; Girolami, V.; Goulson, D.; Kreuzweiser, D. P.; Krupke, C.;
549 Liess, M.; Long, E.; Marzaro, M.; Mitchell, E. a. D.; et al. Environmental fate and exposure;
550 neonicotinoids and fipronil. *Environ. Sci. Pollut. Res.* **2014**, *22* (1), 35–67.

- 551 (27) Jones, A.; Harrington, P.; Turnbull, G. Neonicotinoid concentrations in arable soils after seed
552 treatment applications in preceding years. *Pest Manag. Sci.* **2014**, *70* (12), 1780–1784.
- 553 (28) Gupta, S.; Gajbhiye, V. T.; Gupta, R. K. Soil dissipation and leaching behavior of a
554 neonicotinoid insecticide thiamethoxam. *Bull. Environ. Contam. Toxicol.* **2008**, *80* (5), 431–
555 437.
- 556 (29) Huseeth, A. S.; Groves, R. L. Environmental fate of soil applied neonicotinoid insecticides in an
557 irrigated potato agroecosystem. *PLoS One* **2014**, *9* (5), e97081.
- 558 (30) Pisa, L. W.; Amaral-Rogers, V.; Belzunces, L. P.; Bonmatin, J. M.; Downs, C. a; Goulson, D.;
559 Kreutzweiser, D. P.; Krupke, C.; Liess, M.; McField, M.; et al. Effects of neonicotinoids and
560 fipronil on non-target invertebrates. *Environ. Sci. Pollut. Res. Int.* **2014**, *22* (1), 68–102.
- 561 (31) Tomizawa, M.; Casida, J. E. Neonicotinoid insecticide toxicology: mechanisms of selective
562 action. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 247–268.
- 563 (32) Morrissey, C. a.; Mineau, P.; Devries, J. H.; Sánchez-Bayo, F.; Liess, M.; Cavallaro, M. C.;
564 Liber, K. Neonicotinoid contamination of global surface waters and associated risk to aquatic
565 invertebrates: A review. *Environ. Int.* **2015**, *74*, 291–303.
- 566 (33) Krupke, C. H.; Hunt, G. J.; Eitzer, B. D.; Andino, G.; Given, K. Multiple routes of pesticide
567 exposure for honey bees living near agricultural fields. *PLoS One* **2012**, *7* (1), e29268.
- 568 (34) Stewart, S. D.; Lorenz, G. M.; Catchot, A. L.; Gore, J.; Cook, D.; Skinner, J.; Mueller, T. C.;
569 Johnson, D. R.; Zawislak, J.; Barber, J. Potential Exposure of Pollinators to Neonicotinoid
570 Insecticides from the Use of Insecticide Seed Treatments in the Mid-Southern United States.
571 *Environ. Sci. Technol.* **2014**, *48* (16), 9762–9769.
- 572 (35) Holland, J. M.; Smith, B. M.; Storkey, J.; Lutman, P. J. W.; Aebischer, N. J. Managing
573 habitats on English farmland for insect pollinator conservation. *Biol. Conserv.* **2015**, *182*, 215–
574 222.
- 575 (36) Garbuzov, M.; Couvillon, M. J.; Schürch, R.; Ratnieks, F. L. W. Honey bee dance decoding
576 and pollen-load analysis show limited foraging on spring-flowering oilseed rape, a potential
577 source of neonicotinoid contamination. *Agric. Ecosyst. Environ.* **2015**, *203*, 62–68.
- 578 (37) Riedinger, V.; Renner, M.; Rundlöf, M.; Steffan-Dewenter, I.; Holzschuh, A. Early mass-
579 flowering crops mitigate pollinator dilution in late-flowering crops. *Landsc. Ecol.* **2013**, *29* (3),
580 425–435.
- 581 (38) Bommarco, R.; Marini, L.; Vaissière, B. E. Insect pollination enhances seed yield, quality, and
582 market value in oilseed rape. *Oecologia* **2012**, *169* (4), 1025–1032.
- 583 (39) Stanley, D. a.; Stout, J. C. Pollinator sharing between mass-flowering oilseed rape and co-
584 flowering wild plants: implications for wild plant pollination. *Plant Ecol.* **2014**, *215*, 315–325.
- 585 (40) Mohr, N. A.; Jay, S. C. Nectar- and Pollen-Collecting Behaviour of Honeybees on Canola
586 (*Brassica campestris* L. and *Brassica napus* L.). *J. Apic. Res.* **1988**, *27* (2), 131–136.
- 587 (41) Gupta, S.; Gajbhiye, V. T.; Gupta, R. K. Effect of light on the degradation of two
588 neonicotinoids viz acetamiprid and thiacloprid in soil. *Bull. Environ. Contam. Toxicol.* **2008**,
589 *81* (2), 185–189.

- 590 (42) European Food Safety Authority. Conclusion on the peer review of the pesticide risk
591 assessment for bees for. *EFSA J.* **2013**, *11* (1), 3067.
- 592 (43) Environmental Protection Agency, Office of Pesticide Programs, E. F. and E. D. *White paper*
593 *in support of the proposed risk assessment process for bees*; **2012**.
- 594 (44) Sánchez-Bayo, F.; Goka, K. Pesticide residues and bees - a risk assessment. *PLoS One* **2014**, *9*
595 (4), e94482.
- 596 (45) Sandrock, C.; Tanadini, M.; Tanadini, L. G.; Fauser-Misslin, A.; Potts, S. G.; Neumann, P.
597 Impact of chronic neonicotinoid exposure on honeybee colony performance and queen
598 supersedure. *PLoS One* **2014**, *9* (8), e103592.
- 599 (46) Eckert, C. D.; Winston, M. L.; Ydenberg, R. C. The relationship between population size,
600 amount of brood, and individual foraging behaviour in the honey bee, *Apis mellifera* L.
601 *Oecologia* **1994**, *97* (2), 248–255.
- 602 (47) Beekman, M.; Sumpter, D. J. T.; Seraphides, N.; Ratnieks, F. L.W. Comparing foraging
603 behaviour of small and large honey-bee colonies by decoding waggle dances made by
604 foragers. *Funct. Ecol.* **2004**, *18*, 829–835.
- 605 (48) FERA (The Food and Environment Research Agency). *Effects of neonicotinoid seed*
606 *treatments on bumble bee colonies under field conditions*; Sand Hutton, York YO41 1LZ,
607 **2013**.
- 608 (49) Pywell, R. F.; Warman, E. a.; Hulmes, L.; Hulmes, S.; Nuttall, P.; Sparks, T. H.; Critchley, C.
609 N. R.; Sherwood, A. Effectiveness of new agri-environment schemes in providing foraging
610 resources for bumblebees in intensively farmed landscapes. *Biol. Conserv.* **2006**, *129* (2), 192–
611 206.
- 612 (50) Landis, D. A.; Wratten, S. D.; Gurr, G. M. Habitat management to conserve natural enemies of
613 arthropod pests in agriculture. *Annu. Rev. Entomol.* **2000**, *45*, 175–201.
- 614 (51) Bryden, J.; Gill, R. J.; Mitton, R. A. A.; Raine, N. E.; Jansen, V. A. A. Chronic sublethal stress
615 causes bee colony failure. *Ecol. Lett.* **2013**, *16* (12), 1463–1469.

616

617

618

619

620

621

622

623

624

625

626

627 **Fig. 1.** Levels of thiamethoxam, clothianidin, thiacloprid and total neonicotinoids (TMX,
628 CLO, IMD and THC) in pollen collected from OSR flowers and wildflowers from OSR field
629 margins (Black horizontal bars inside boxplots are median values; upper and lower whiskers
630 represent scores outside the middle 50%; open circles represent mild outliers and asterisks are
631 extreme outliers).

632

633

634

635

636

637

638

639

640

641

642

643

644

645

646

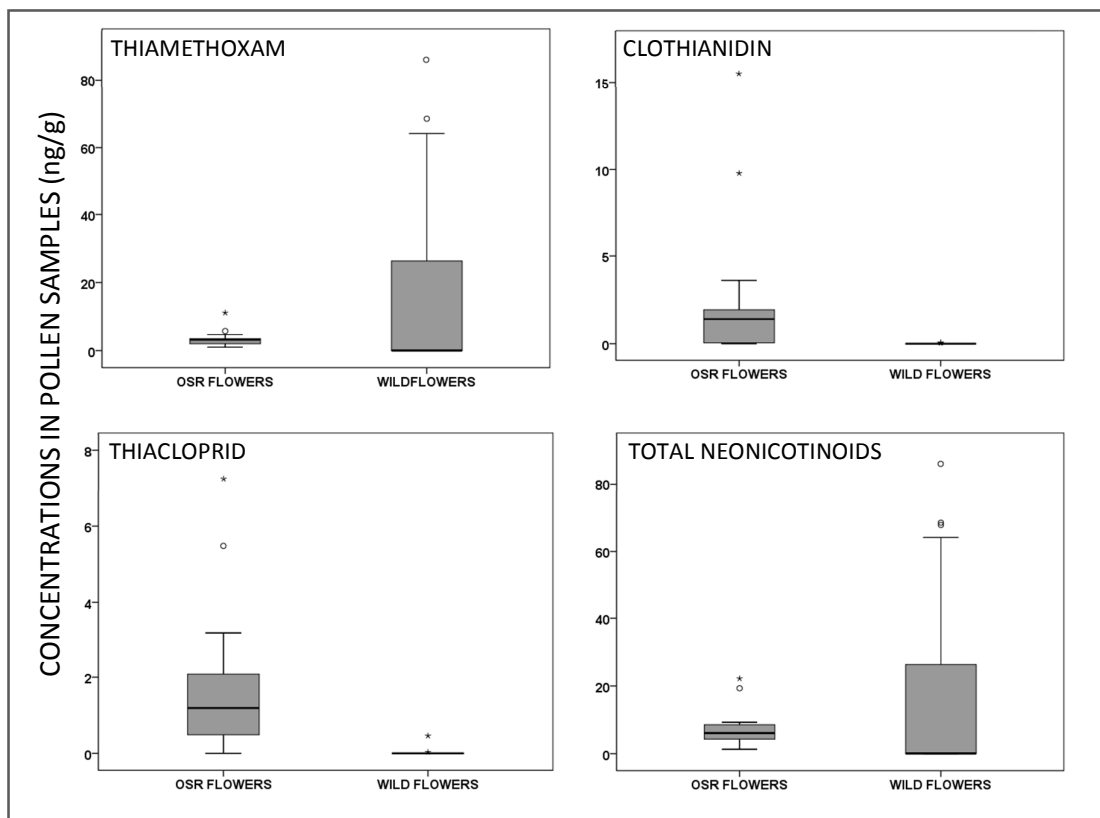
647

648

649

650

651



652 **Fig. 2.** Mean levels of thiamethoxam, imidacloprid, thiacloprid and total neonicotinoid
653 residues detected in hand collected pollen from the wildflowers present in the margins of
654 OSR and WW fields and the mean levels in corbicular pollen of wildflower origin trapped in
655 honey bee hives located in the vicinity of the same fields (Standard error bars are represented
656 in the graphs, and statistically significant differences ($P < 0.05$) are marked with an asterisk).

657

658

659

660

661

662

663

664

665

666

667

668

669

670

671

672

673

674

675

676

677

678

679

680

681

682

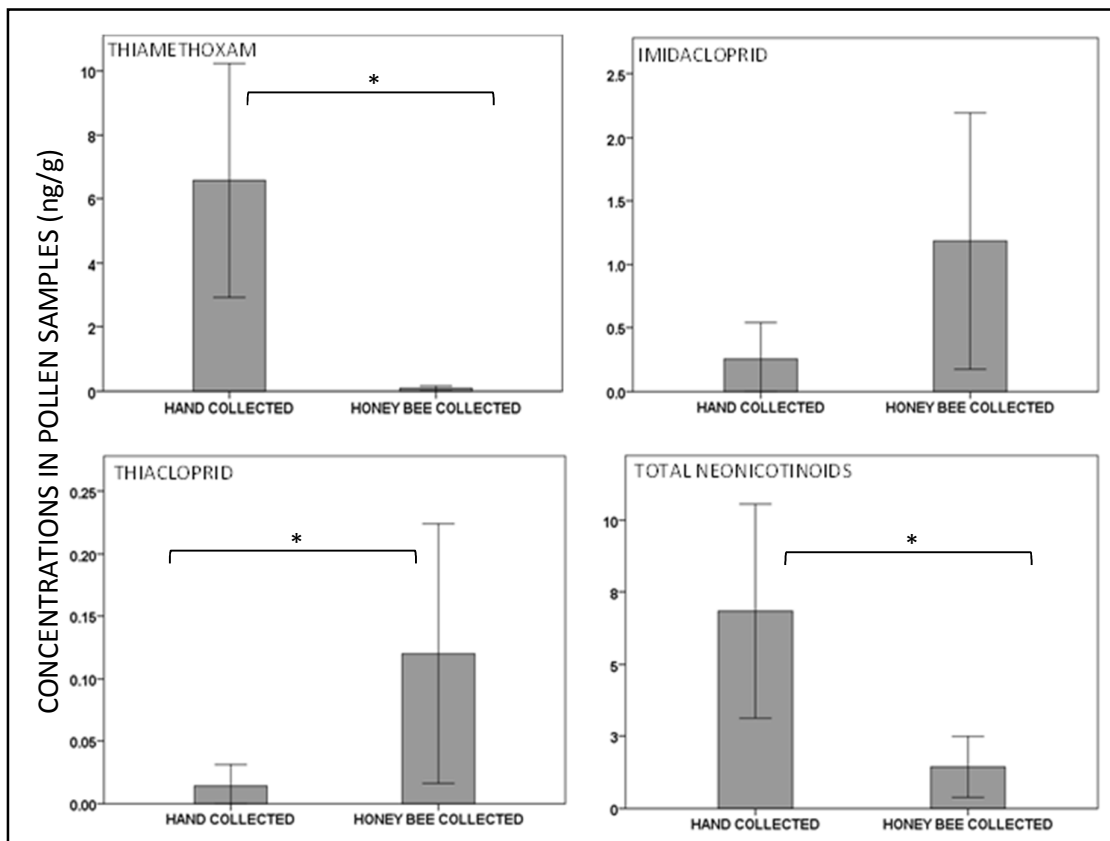
683

684

685

686

687



676

677

678

679

680

681

682

683

684

685

686

687

688 **Table 1.** Number of samples analysed, percentage with detectable levels of neonicotinoid
 689 insecticides, range, mean (\pm Standard Deviation) and median of the levels found in soil
 690 samples collected from oilseed rape (OSR) cropland and field margins (where the seeds were
 691 treated with thiamethoxam at an application rate of 4.2 g a.i. thiamethoxam per kg seed), and
 692 from the field margins of winter wheat crops (WW, where the wheat seeds were treated with
 693 clothianidin at an application rate of 0.5 g a.i. clothianidin per kg seed). All fields were sowed
 694 with harrow power drill combination.

695

ORIGIN OF SOIL SAMPLES	N		TMX	CLO	IMC	THC
		<i>Method detection limit (MDL)(ng/g)</i>	0.04	0.07	0.07	0.01
		<i>Method quantification limit (MQL)(ng/g)</i>	0.12	0.20	0.20	0.04
		FREQUENCY OF DETECTIONS (%)	100%	100%	100%	42.86%
OSR CROPLAND	21	RANGE (ng/g)	0.49 - 9.75	5.10 - 28.6	0.74 - 7.90	$\leq 0.01 - 0.22$
		MEAN \pm S.D. (ng/g)	3.46 ± 2.98	13.28 ± 5.73	3.03 ± 2.05	0.04 ± 0.07
		MEDIAN (ng/g)	2.43	13.05	2.10	≤ 0.01
		FREQUENCY OF DETECTIONS (%)	100%	100%	93.75%	25%
OSR FIELD MARGINS	16	RANGE (ng/g)	0.28 - 1.76	2.25 - 13.33	$\leq 0.07 - 7.17$	$\leq 0.01 - 0.13$
		MEAN \pm S.D. (ng/g)	0.72 ± 0.44	6.57 ± 3.12	1.92 ± 2.06	≤ 0.01
		MEDIAN (ng/g)	0.59	5.61	0.70	≤ 0.01
		FREQUENCY OF DETECTIONS (%)	50%	100%	75%	25%
WW FIELD MARGINS	8	RANGE (ng/g)	$\leq 0.04 - 0.45$	0.41 - 19.12	$\leq 0.07 - 6.30$	$\leq 0.01 - 0.13$
		MEAN \pm S.D. (ng/g)	0.18 ± 0.21	7.71 ± 6.9	1.36 ± 2.19	≤ 0.01
		MEDIAN (ng/g)	≤ 0.12	7.36	0.48	≤ 0.01
		FREQUENCY OF DETECTIONS (%)	50%	100%	75%	25%

696

697

698

699

700

701

702

703

704

705

706

707

708

709

710

711 **Table 2.** Number of samples analysed, frequency of detections, range, mean (\pm Standard
 712 Deviation) and median of levels found and in pollen and nectar samples collected from
 713 oilseed rape (OSR) flowers (7 fields) and from wildflowers collected from the margins of 5
 714 OSR and 5 winter wheat (WW) fields, and pollen collected by honey bees.

715 * only one sample with detectable levels of this compound.

716

ORIGIN OF POLLEN SAMPLES		N		TMX	CLO	IMC	THC
			<i>Method detection limit (MDL)(ng/g)</i>	0.12	0.12	0.16	0.04
			<i>Method quantification limit (MQL)(ng/g)</i>	0.36	0.36	0.48	0.12
POLLEN	OSR FLOWERS	21	FREQUENCY OF DETECTIONS (%)	100%	90.5%	0%	85.7%
			RANGE (ng/g)	1.02-11.10	≤ 0.12 -14.50	≤ 0.16	≤ 0.04 -7.25
			MEAN \pm S.D. (ng/g)	3.26 \pm 2.16	2.27 \pm 3.52		1.68 \pm 1.84
			MEDIAN (ng/g)	3.16	1.40		1.19
	WILDFLOWERS FROM OSR MARGIN	43	FREQUENCY OF DETECTIONS (%)	58.1%	14%	11.6%	4.7%
			RANGE (ng/g)	≤ 0.12 -86.02	≤ 0.12 - ≤ 0.36	≤ 0.16 -12.29	≤ 0.04 -0.46
			MEAN \pm S.D. (ng/g)	14.81 \pm 25.17		0.56 \pm 2.10	≤ 0.04
			MEDIAN (ng/g)	≤ 0.36		≤ 0.16	≤ 0.04
	WILDFLOWERS FROM WW MARGIN	55	FREQUENCY OF DETECTIONS (%)	1.8%	0%	3.6%	3.6%
			RANGE (ng/g)	≤ 0.12 -7.47*	≤ 0.12	≤ 0.16 -0.58	≤ 0.04 -0.64
			MEAN \pm S.D. (ng/g)	0.14 \pm 1.01		≤ 0.16	≤ 0.04
			MEDIAN (ng/g)	≤ 0.12		≤ 0.16	≤ 0.04
	COLLECTED BY HONEY BEES DURING OSR BLOOM (JUNE)	34	FREQUENCY OF DETECTIONS (%)	50%	23.5%	20.6%	58.8%
			RANGE (ng/g)	≤ 0.12 -1.81	≤ 0.12 -1.12	≤ 0.16 -25.55	≤ 0.04 -2.77
			MEAN \pm S.D. (ng/g)	0.20 \pm 0.44	≤ 0.12	2.51 \pm 6.28	0.30 \pm 0.65
			MEDIAN (ng/g)	≤ 0.12	≤ 0.12	≤ 0.16	≤ 0.12
	COLLECTED BY HONEY BEES AFTER OSR BLOOM (AUGUST)	46	FREQUENCY OF DETECTIONS (%)	43.5%	4.3%	15.2%	19.6%
			RANGE (ng/g)	≤ 0.12 -0.31	≤ 0.12 -0.28	≤ 0.16 -2.52	≤ 0.04
MEAN \pm S.D. (ng/g)			≤ 0.12	≤ 0.12	≤ 0.16		
MEDIAN (ng/g)			≤ 0.12	≤ 0.12	≤ 0.16		
ORIGIN OF NECTAR SAMPLES		N		TMX	CLO	IMC	THC
			<i>Method detection limit (MDL)(ng/g)</i>	0.10	0.17	0.17	0.03
			<i>Method quantification limit (MQL)(ng/g)</i>	0.30	0.50	0.50	0.08
NECTAR	OSR FLOWERS	13	FREQUENCY OF DETECTIONS (%)	53.9%	30.8%	0%	53.9%
			RANGE (ng/g)	≤ 0.10 -13.30	≤ 0.17 -13.24	≤ 0.17	≤ 0.03 -1.23
			MEAN \pm S.D. (ng/g)	3.20 \pm 4.61	2.18 \pm 3.99		0.26 \pm 0.36
			MEDIAN (ng/g)	≤ 0.10	≤ 0.17		0.11
	WILDFLOWERS FROM OSR MARGIN	24	FREQUENCY OF DETECTIONS (%)	20.8%	20.8%	0%	25%
			RANGE (ng/g)	≤ 0.10 -1.80	≤ 0.17 - ≤ 0.50	≤ 0.17	≤ 0.03 - ≤ 0.08
			MEAN \pm S.D. (ng/g)	0.10 \pm 0.37			
			MEDIAN (ng/g)	≤ 0.10			
	WILDFLOWERS FROM WW MARGIN	8	FREQUENCY OF DETECTIONS (%)	0%	0%	0%	0%
			RANGE (ng/g)	≤ 0.10	≤ 0.17	≤ 0.17	≤ 0.03
			MEAN \pm S.D. (ng/g)				
			MEDIAN (ng/g)				

717

718

719

720

721

722