

THE BEES' BURDEN

AN ANALYSIS OF PESTICIDE RESIDUES IN COMB POLLEN (BEEBREAD) AND TRAPPED POLLEN FROM HONEY BEES (*APIS MELLIFERA*) IN 12 EUROPEAN COUNTRIES

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An analysis of pesticide residues in comb pollen (beebread) and trapped pollen from honey bees (*Apis mellifera*) in 12 European countries

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EXECUTIVE SUMMARY



Germany: Dirk
Zimmerman, a
Greenpeace campaigner
for sustainable
agriculture, and
beekeeper Dr Simon
Bach observe bees
entering a hive.

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This study reports concentrations of pesticides found in pollen brought back to hives by foraging bees, and sampled using pollen traps (trapped pollen) or direct from the comb (comb pollen, beebread). Twenty-five samples of comb pollen stored over winter from the 2012 foraging season were obtained from locations in seven European countries, and subsequently 107 samples of trapped pollen from the 2013 foraging season were obtained from locations in 12 European countries and analysed at an accredited laboratory. In terms of the geographical areas covered, and the numbers of samples taken simultaneously, this is one of the most extensive studies of pesticides in bee-collected pollen carried out to date.

Residues of at least one of 53 pesticides (including 22 insecticides/acaricides, 29 fungicides and two herbicides) were identified in 72 of the 107 trapped pollen samples, while residues of at least one of 17 pesticides (including nine insecticides/acaricides and eight fungicides) were identified in 17 of the 25 samples of comb pollen (beebread).

The results indicate the widespread use of the insecticides chlorpyrifos (in 18 samples) and thiacloprid (14 samples), as well as the fungicide boscalid (14 samples), which were the most commonly detected residues in trapped pollen samples. The results also indicate that a wide variety of plant protection products, particularly fungicides, were present in the trapped pollen, with a maximum of 17 different residues (three insecticides/acaricides and 14 fungicides) detected in a sample from Italy. Overall, the results reported here are broadly consistent with other studies of trapped pollen and bee products, in which a wide variety of pesticides were also commonly detected. This study sheds further light on the potentially serious toxic exposures suffered by honey bees at an individual and colony level throughout their lifecycle, and raises significant questions about likely exposures of wild bee populations and other wild pollinators to chemicals through various pathways. These exposures have either been ignored or have been underestimated in past and current discussions of bee health and pollinator protection measures.



The exposure of bees and bee larvae to mixtures of pesticides is of significance because recent research has established that some components of the mixture are capable of interacting in a synergistic manner, such that the mixture proves more toxic than its individual components. Mixtures identified as being of potential concern in this regard include those containing in-hive acaricidal treatments, together with fungicides that work through sterol biosynthesis inhibition (SBI). This class of fungicides is well represented in the samples reported here, raising the possibility that the mixtures reported might be toxicologically active to bees exposed to them.

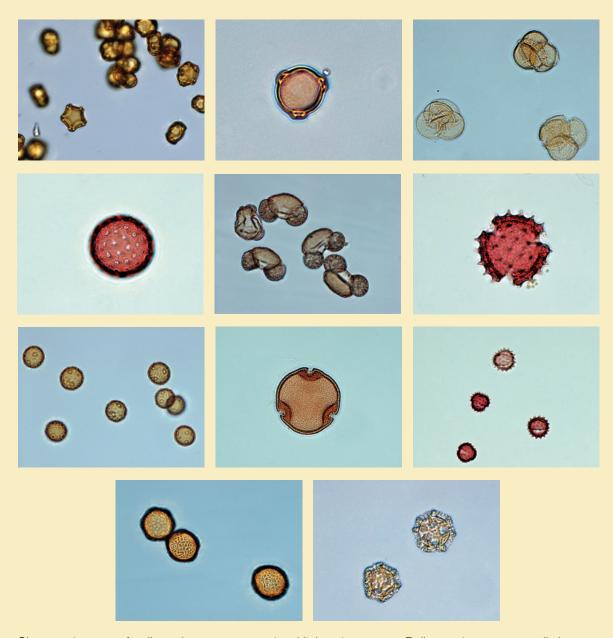
Progress towards eliminating pesticide exposure of bees through foraging has been limited to date. Partial bans have been emplaced on the use of the systemic insecticides imidacloprid, thiamethoxam, clothianidin and fipronil in seed dressings, and in the ground and leaf treatment of certain specified crops. In order to confer greater protection to wild and cultured pollinators, this ban should be made permanent, and expanded in scope to include other uses and other pesticides. There is a need to ensure, through research and through the application of holistic assessment, that pesticides with bee-harming properties are not permitted for use. In addition, it is important that existing products are not simply replaced by other pesticides that might not have been fully evaluated. Thiacloprid, for example, was found quite frequently in trapped pollen in the current study, indicating widespread use in Europe during 2013, and its possible use to replace the restricted neonicotinoids. In addition, other insecticides known to be very harmful to bees should also be brought under strictest possible control. This should include chlorpyrifos (found frequently in this study), together with the synthetic pyrethoids cypermethrin and deltamethrin.

The results of this study, considered together with other work reported in the scientific literature, indicate that current regulation of pesticides, based upon a limited number of environmental properties and toxicity values reported for individual active substances, may not be adequately protective of pollinator populations. Surveillance monitoring of pesticides to which pollinators are exposed needs to address the widest possible spectrum of substances (and their metabolites) that can be resolved using state-of-the -art analytical methods at the best achievable detection limits. In addition, the exposure of pollinators to pesticides as mixtures needs to be taken fully into account, particularly the possibility of synergistic interactions, which are difficult to predict quantitatively using many of the currently available models of joint toxicity. Accordingly, strategies should also be developed that aim at, and result in, a substantial reduction in use of pesticides of all types as a precautionary goal in itself. To afford a high level of protection to pollinator populations, coordinated Bee Action Plans need to be established. In addition to more effective regulation and control of agricultural chemicals, such plans should include the monitoring of the health of bees and other pollinators. They should also work to improve the conservation of natural and semi-natural habitats around agricultural landscapes, as well as enhancing biodiversity within agricultural fields.

Finally, funding should be radically increased for research and development of ecological farming practices, which move away from reliance on chemical pest control towards biodiversity-based tools to manage pests and enhance ecosystem health. EU policy makers should direct more funding for ecological agriculture solutions research under the auspices of the CAP (direct payments) and Horizon 2020 (EU research) programmes.

Overall, therefore, this study points to the need to progressively reduce and eliminate the exposure of bees to the cocktail of toxic agrochemicals to which they may be exposed throughout their lifecycle, and to move towards ecological farming methods.

A world of pollen



Close-up images of pollen taken on a transmitted light microscope. Pollen grains are generally between 15-100 microns in diameter. Images courtesy of Mark Grosvenor, University of Exeter.

From top row, left-to-right: Alnus glutenosa (European alder); Betula pendula (silver birch); Calluna vulgaris (common heather); Chenopodium album (white goosefoot); Pinus sylvestris (Scots pine); Cirsium arvense (creeping thistle); Plantago lanceolata (ribwort plantain); Tilia vulgaris (lime tree); Senecio vulgaris (common groundsel); Ulmus glabra (wych elm); and Taraxacum vulgaris (dandelion).



INTRODUCTION



Northern Italy: Bees in hives belonging to beekeeper Francesca Zacchetti.

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Modern agriculture relies on various synthetic chemical inputs, ranging from inorganic fertilisers through to toxic pesticides, designed to address insect and fungal pests as well as to control weed plant species. The potential scale of chemical interventions over a single growing season is exemplified in thought-provoking data on crops grown in the UK and published online (Goulson 2014). Beginning with seed dressing containing insecticide and fungicide, a single crop of winter oilseed rape was treated with some 20 individual biocides, together with inorganic fertilisers, at various points in time. In the case of a winter wheat crop, some 18 different biocides were applied, together with inorganic fertilisers, over the growing season. While some of these chemical interventions are made at a time when bees may not be foraging, others have the potential to expose foraging bees directly to a mixture of pesticides.

The European Food Safety Authority (EFSA 2014) has noted that, overall at the European level, there are significant gaps in knowledge of the multiple stressors affecting both wild and cultured pollinators, including the impacts of mixtures of pesticides. The report notes *inter alia* the need to devise, through coordinated research, common methods of monitoring bees and of hazard identification for different classes of chemicals. The EFSA review is timely. The impact of agrochemicals on bee populations (and indeed, populations of other insect pollinators) is attracting increasing attention.

Greenpeace has recently published a review of the factors thought to be contributing to the marked decline in honey bee and wild pollinator populations (Tirado *et al.* 2013). As the report *Bees in Decline* makes clear, the observed overall global decline in bee populations and in their health cannot be attributed to any single factor. Much of the work to date has focussed on the honey bee, *Apis mellifera*, and the most important factors so far identified relate to parasitic, bacterial and viral disease, as well as to wider agricultural practices that can impact at many points in the life cycle of bees. In short, the decline is undoubtedly the product of multiple factors – both known and unknown – acting singly or in combination.

The parasitic mite *Varroa destructor* is a globally significant threat to bees, while the microsporidian parasite *Nosema ceranae* is significant in regions such as eastern and southern Europe. There is evidence that exposure to some pesticides may compromise the immune system of bees. In addition, habitat destruction and the fragmentation of natural and semi-natural habitats, with concomitant expansion of monocultured crops and reduction in wild plant diversity, can all play a role in challenging the ability of pollinator populations to remain viable. In addition to all these factors, erratic weather patterns driven by climate change may also emerge as a highly important factor, though at present such impacts are difficult to characterise, predict or attribute.

In the face of the diverse driving factors of pollinator decline, Bees in Decline concluded that one crucial first step would be to ban the use of several pesticides with a known high toxicity to bees. The list included imidacloprid, thiamethoxam, clothianidin, fipronil, chlorpyrifos, cypermethrin and deltamethrin. In April 2013, a majority of EU countries supported the European Commission proposal (European Commission 2013a) to temporarily restrict the use of three pesticides in some applications: imidacloprid, clothianidin and thiamethoxam. Partial bans of neonicotinoids were already in place in Italy, France, Germany and Slovenia. In Italy, no significant negative impacts on agricultural production were reported as a result of the national ban, but there were some reported positive effects on the health of bees (European Parliament 2012). The restrictions placed on the three neonicotinoids were followed by a review of fipronil (EFSA 2013), which identified a high risk to bees from dust generated from use of fipronil-treated seeds, as well as significant data gaps related to exposure pathways through pollen, nectar and guttation fluid. This led, in turn, to European Commission Implementing Regulation (EU) 781/2013, which prohibited the use of fipronil in seed dressings (European Commission 2013b), effective from December 31, 2013.

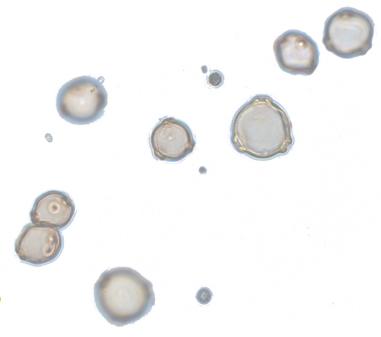
All of the insecticides restricted in Europe for use in seed dressings are systemic or partly systemic pesticides, which enter the plant and are translocated to all parts of it. Since this can include the pollen and nectar (see, for example, Dively & Kamel 2012, Pohorecka et al. 2012), there is a direct risk posed to bees that collect both materials and transport them back to the hive. The problem, however, is not confined to systemic pesticides alone. An early study in France reported 19 pesticide residues in bee-collected pollen from a variety of sites (Chauzat et al. 2006), while Lambert et al. (2013) reported 23 pesticides present in pollen samples taken in western France. Skerl (2009) reported data on beebread and bee-collected pollen from treated apple orchards in Slovenia, indicating that applied insecticides and fungicides were found in pollen in the hive some days after application. A study in the USA, conducted at multiple locations, examined bee-collected pollen in the form of beebread and trapped pollen. Almost 100 different pesticides and metabolites were reported in total in this matrix, from 350 samples, while contamination was also found in the wax (Mullin et al. 2010).

One recent study (Stoner & Eitzer 2013) detected 60 individual pesticides or their metabolites from various chemical classes in bee-collected pollen sampled over 2 to 5 years at a variety of sites in Connecticut in the USA. A similar study of pooled samples was carried out on bee-collected pollen in key fruit crop growing areas in the USA, although the researchers noted that pollen in some of these areas came predominantly from wild plants rather than from the fruit plants themselves. Even so, 35 individual pesticides were detected, among which fungicides in particular were present at high loadings (Pettis *et al.* 2013). In a screening study of 14 honey bee colonies in southern Sweden, 26 individual pesticides were detected in comb pollen, with up to 13 detected in individual samples (Jonsson & Krueger 2013). The highest concentrations recorded were for two fungicides, namely azoxystrobin and prochloraz.

A German national project analysed samples of beebread collected between 2005 and 2006, and isolated a total of 42 active ingredients from 105 samples, some having more than one pesticide present, but with 25 samples containing nothing above detection limits. Samples taken in 2007 were broadly comparable, with 42 active ingredients detected in 110 beebread samples, although the specific pesticides found showed some differences (Genersch *et al.* 2010).

Several of the above mentioned studies also analysed honey, beeswax and even individual bees, and reported a similar diversity of pesticides to be present. This was also true of a study of these matrices carried out in Belgium (Nguyen et al. 2009). Bernal et al. (2010) reported 54 active ingredients in 42% of spring-sampled stored pollen from Spain, while in autumn-stored pollen, 14 active ingredients were reported from 31% of samples. The relative percentage of samples that contained residues in relation to the total varied significantly across the different regions from which samples were obtained.

The analyses reported here were carried out on 25 samples of beebread stored during the 2012 foraging season and overwintered into 2013, collected from various locations in seven European countries, as well as 107 samples of bee-foraged pollen collected in pollen traps during the 2013 foraging season from locations in 12 European countries. The aim of the study was to identify and quantify the pesticides present in these bee products.





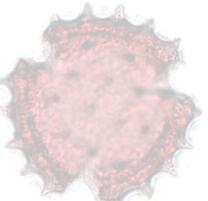
METHODS AND MATERIALS



Close-up of a honey bee with pollen. © Fred Dott / Greenpeace

The study was carried out in two phases. The first phase involved the sampling and analysis of beebread from a number of hives located in various European countries, and this initial work was followed up with the analysis of samples of bee-collected pollen (with some further beebread) samples from various European locations. Analysis of both matrices was carried out at an independent laboratory in Germany, using a common analytical protocol (QuEChERS) designed for the analysis of food materials and suitably adapted (Lesuer *et al.* 2008). The neonicotinoid pesticides clothianidin, thiamethoxam and imidacloprid were analysed using a targeted LCMS/MS analysis, with a detection limit (LOD) of 0.3μ g/kg and a limit of quantitation (LOQ) of 1μ g/kg. All other pesticides were analysed using a multiresidue GC-MS/MS and LC-MS/MS method covering 300 different substances, with a detection limit (LOD) of 3μ g/kg and an LOQ of 10μ g/kg in most cases. Standard solutions were prepared in-house from individual compounds (Dr. Ehrenstorfer, Sigma-Aldrich).

Broadly, 10ml of deionised water (Barnstead, Nanopure DiamondTM, Thermo Scientific) was added to approximately 5g of bee-collected pollen, or to beebread extracted directly from the comb and accurately weighed into a Teflon centrifuge tube. 10ml of acetonitrile (HPLC Gradient Grade, VWR) was added, together with an internal standard solution (containing isoproturon-d6 for LC-MS/MS analysis, and anthracene-d10 for GC-MS analysis). To this mixture was added 4g of anhydrous magnesium sulphate, 1g of sodium chloride, 1g of trisodium citrate dihydrate, and 0.5g of disodium hydrogen citrate sesquihydrate and the whole mixed with a vortex mixer for 10 minutes. The mixture was then centrifuged for 13 minutes at 10,000rpm in a Rotina 380 R refrigerated centrifuge, and 7ml of the supernatant was then transferred to a tube containing 1g of anhydrous magnesium sulphate and was then briefly shaken by hand and centrifuged again. 1ml of the supernatant was then removed for LC-MS/MS analysis.



300mg of PSA as a sorbent were added to the remaining 6ml of solution, and the mixture was then shaken by hand for one minute and then centrifuged for 13 minutes at 10,000rpm in a Rotina 380 R refrigerated centrifuge Two aliquots of 0.2ml of the supernatant were then transferred to two vials, and 10μ l per ml of extract of 5% formic acid solution in acetonitrile were added as an analyte protectant/stabilisation solution. These aliquots were used for GC-MS/MS and for GC-MSD analyses.

The following analytical instruments were used for the analyses.

LC-MS/MS system

Autosampler: Finnigan Surveyor Autosampler Plus (Fa. ThermoFisher)

LC-Pump: Finnigan Surveyor MS Pump Plus (Fa. ThermoFisher)

Mass Spectrometer: TSQ Quantum Ultra (Fa. ThermoFisher)

Software: Thermo Xcalibur 2.1/TraceFinder 2.1

Samples were analysed using a Finnigan Surveyor autosampler and liquid chromatography system coupled to a ThermoFisher triple quadrupole mass spectrometer (TSQ Quantum Ultra). Samples were injected onto a 150 x 2.1mm 5µm, Thermo Hypersil Gold reversed-phase column (ThermoFisher, San Jose, CA, USA). 20µl of sample were injected and the separations were performed at 30°C. The analytes were eluted from the column with a gradient flow (0.2ml/min) of 0.01% glacial acetic acid plus 5mmol ammonium acetate in water (mobile phase A) and 0.01% glacial acetic acid plus 5mmol ammonium acetate in methanol (mobile phase B). The gradient was held at 90% mobile phase A for one minute, before being ramped down to 20% over 9 minutes. This condition for elution was held for 10 minutes. The gradient was set back to start conditions, and re-equilibrated for four minutes in preparation for the next sample injection.

For the targeted analysis of neonicotinoids, the equipment was the same but specific scan events were used. The separations were performed with a higher flow rate of 0.3ml/min. The gradient elution programme started with 80% A, and was changed linearly to 20% A between 1 and 6 minutes, and was then held for 4 minutes.

Mass spectrometric data were collected in the positive heated-ESI-mode (electrospray ionisation). The capillary temperature was 300°C at a spray voltage of 4.0kV. The sheath gas pressure was 25arb and the aux gas 12arb respectively.



GC-MS/MS (QQQ)

Autosampler: MPS2 incl. ALEX (Gerstel)Gas chromatograph: 7890 (Agilent Technologies)

Mass Spectrometer: 7000 Triple Quadrupol (Agilent Technologies)

Software: MassHunter Workstation Version B.04.00; MassHunter Aquisition

Version B.05.00.412; Gerstel Maestro 1.3.10.3

GC-MS/MS analysis was performed on a GC 7890 gas chromatograph (Agilent) connected to a 7000 Triple Quadrupole mass spectrometer. Separations were achieved on a $30 \, \mathrm{m} \times 0.25 \, \mathrm{mm}$ 0.25 μ m Agilent HP-5ms capillary column. Initial PTV inlet temperature was 80°C and was then programme-controlled raised to 250°C. Injections were conducted in the solvent vent mode. The injection volume was 4μ l. Initial conditions for helium carrier gas pressure were 27.5psi. The initial oven temperature was 70°C for 2 minutes. The oven was then heated at 25°C/min to 150°C, then at 3°C/min to 200°C, then at 8°C/min, and then to a final temperature of 280°C, at which it was held for 10 minutes.

GC-MS (MSD)

Autosampler: MPS2 incl. ALEX (Gerstel)

Gas chromatograph: GC 6890N (Agilent Technologies)

Mass spectrometer: MS 5975 XL inert (Agilent Technologies)

Software: Enhanced Data Analysis/MSD Chemstation D.02.00.275

Gerstel Maestro 1.3.8.14

GC-MSD analysis was performed on a GC 6890N gas chromatograph (Agilent) connected to a MS 5975 XL mass-selective detector. Separations were achieved on a 30m x 0.25mm 0.25 μ m Varian VF-5ms capillary column. Injection port initial temperature was 70°C and was then programme-controlled raised to 250°C. The injection volume was 1 μ l. Initial conditions for helium carrier gas flow rate were 1.0ml/min. The initial oven temperature was 120°C and was held for 1 minute. The oven was heated at 4°C/min to a final temperature of 280°C, and was then held for 4 minutes.



RESULTS



A bee on a cosmos flower.

© Axel Kirchhof / Greenpeace

Residues of at least one of 53 pesticides (including 22 insecticides/acaricides, 29 fungicides and two herbicides) were identified in 72 of the 107 trapped pollen samples, while residues of at least one of 17 pesticides (including nine insecticides/acaricides and eight fungicides) were identified in 17 of the 25 samples of comb pollen (beebread).

The most commonly detected residues in trapped pollen were of the organophosphate insecticide/acaricide chlorpyrifos-ethyl. This was found in 18 out of 107 samples, including six out of seven samples from Poland (10-119 μ g/kg) and in five of 14 samples from Spain (11-705 μ g/kg). Chlorpyrifos-methyl was found in one sample from Italy. Chlorpyrifos is one of the seven bee-harming pesticides identified as candidates for priority rapid phase-out by Tirado *et al* (2013).

Boscalid, a carboxamide fungicide, was detected in 14 samples, including five of the 15 samples taken in Germany (12-144 μ g/kg), though by far the highest concentration (1081 μ g/kg) was identified in one of two pollen samples from Sweden. The neonicotinoid insecticide thiacloprid was also found in 14 samples, including eight of the 15 taken in Germany, at concentrations of 10-250 μ g/kg. Dimethomorph (a morpholine-class fungicide derived from cinnamic acid) was detected in 11 samples (11 of the 12 samples from Italy at 204-1273 μ g/kg).

In samples of comb pollen (beebread), the most frequently encountered pesticide was the insecticide/acaricide Amitraz (six of 25 samples, including four of the five samples from Switzerland at 31-177 μ g/kg), followed by tau-fluvalinate, a synthetic pyrethroid insecticide/acaricide detected in four samples, including all three taken in Spain (11-13 μ g/kg), while coumaphos, a phosphorothionate insecticide/acaricide was found in two samples, both from Spain (204-1273 μ g/kg).

Other active ingredients found relatively frequently in pollen were fungicides. Fenhexamid, trifloxystrobin and folpet and were each found in nine samples; spiroxamine and thiophanate-methyl in eight samples; while iprovalicarb and cyprodinil were each detected in seven samples, either alone or in combination .

Of the three neonicotinoid insecticides currently subject to restrictions on use in Europe, imidacloprid was found in six of the 107 pollen samples (5.6%). These comprised four samples from Spain (7.6-148.5 μ g/kg) and two samples from Italy (1.7-11.0 μ g/kg). Clothianidin was found in two samples (1.8%) (one from Austria at 4.7 μ g/kg, and one from Sweden at 1.8 μ g/kg). Thiamethoxam residues were not detected in any of the pollen samples. None of the beebread samples analysed contained detectable levels of any of the banned neonicotinoids.

By far the widest range of active ingredients were associated with samples of pollen from Italy, especially those collected in the vicinity of vine culture. For example, residues of 17 different pesticides (including 14 fungicides and three insecticides/acaricides) were detected in pollen collected in the vicinity of vineyards close to the Cisterna d'Asti, in the Valle S Matteo, on June 16, 2013, while 12 residues (10 fungicides and two insecticides/acaricides) were identified in a sample collected from the Montebelluna region on June 27, 2013.

In the case of beebread, the greatest number of residues were found in a sample collected from a stored frame in the Gilena region of Andalucia in Spain in March 2013, though in this case residues of insecticides/acaricides (six active ingredients) were more conspicuous than those of fungicides (one active ingredient).

In addition, the commonly used insect repellent chemical DEET (diethyl toluamide) was found in one sample of trapped pollen and in one beebread sample. This may have been due to use by the beekeeper as a personal insect repellent, and it is therefore not counted in the summary statistics (i.e. total numbers of pesticides found) reported above. Similarly, piperonyl butoxide, a synergist used to increase the toxicity of a number of insecticides – principally carbamates, pyrethroids and rotenone – was found in three pollen samples, but is similarly not counted in the descriptive statistics. Pentachloroanisole, a degradation product of both of the fungicides pentachlorophenol and pentachloronitrobenzene and identified in one of the beebread samples, has also been excluded from the summary statistics above.

The data are summarised in the following Tables 1-4, while the full data set is reproduced in Appendix 1.



Country	Sampling period, 2013	Number of samples	Key pesticides (banned neonicotinoids and other frequently encountered pesticides*) (Number of samples in which found) [Contentration range in $\mu g/kg$]
Austria	May	3	Clothianidin (1) [4.7], Thiacloprid (1) [24], Tebuconazole (1) [30]
France	Apr-Sep	12	Boscalid (2) [48-269], Folpet (1) [11], Tebuconazole (1) [159], Thiophanate-methyl (1) [24]
Germany	May-Jun	15	Thiacloprid (8) [10-250], Amitraz (incl. metabolites) (1) [11], Azoxystrobin (2) [30-69], Boscalid (5) [12-144], Cyprodinil (2) [454-590], Fenhexamid (1) [2550], Spiroxamine (1) [10], Thiophanate-methyl (1) [17], Trifloxystrobin (2) [26-1104]
Greece	Jun-Jul	10	Amitraz (2) [20-33], Chlorpyrifos-ethyl (1) [360]
Hungary	May-Jul	7	Thiacloprid (3) [22-33], Amitraz (incl. metabolites) (4) [13-46], Boscalid (2) [18-57], Chlorpyrifos-ethyl (1) [123], Fenhexamid (1) [13], Folpet (1) [97]
Italy	May-Jul	12	Imidacloprid (2) [1.7-11], Chlorpyrifos-ethyl (3) [10-562], Boscalid (3) [13-43], Cyprodinil (2) [22-146], Dimethomorph (11) [20-2045], Fenhexamid (6) [11-43], Folpet (6) [10-1316], Iprovalicarb (7) [11-320], Metalaxyl/Metalaxyl-M (6) [12-454], Spiroxamine (7) [12-83], Tebuconazole (3) [22-296], Thiophanate-methyl (1) [29], Trifloxystrobin (7) [22-220]
Luxembourg	May-Jun	5	No pesticides detected
Poland	May-Jun	7	Thiacloprid (1) [147], Chlorpyrifos-ethyl (6) [10-119], Azoxystrobin (3) [17-22], Tebuconazole (1) [16], Thiophanatemethyl (2) [10-68]
Romania	Jun-Aug	10	Azoxystrobin (1) [18], Fenhexamid (1) [13], Folpet (1) [51], Thiophanate-methyl (2) [27-93]
Spain	Jul-Aug	14	Imidacloprid (4) [7.6-148.5], Chlorpyrifos-ethyl (5) [11-705]
Sweden	Jul	2	Clothianidin (1) [1.8], Boscalid (2) [147-1081]
Switzerland	Apr-Sep	10	Thiacloprid (1) [31], Cyprodinil (2) [91-10169], Thiophanatemethyl (1) [21]

^{*}residues found in 6 or more samples of the total set of 107 samples of pollen

Table 1: Key pesticides found in six or more samples of trapped pollen, along with concentration ranges reported for each country from which the pollen samples were obtained. The sampling period within which samples were obtained in each country is shown in column 2.

Class/Type:

CAR = carbamate, FORM = formamidine, FUNG = fungicide, HERB = herbicide, INS = misc. insecticide, NEO = neonicotinoid, OC = organochlorine, OP = organophosphate, PS = partial systemic, PYR = pyrethroid, REP = insect repellent, S = systemic, SBI = Steroid Biosynthesis Inhibitor, SYN = synergist

Pesticide	Class/Type	Frequency of		Countries in which found
		detection	1	(Number of samples)
				[Concentration range in µg/kg]
		Samples	% samples	
Chlorpyrifos (-ethyl)	OP	18	16.8	France (1/12) [10], Greece (1/10) [360], Hungary (1/7) [123], Italy (3/12) [10-562], Poland (6/7) [10-119], Spain (5/14) [11-705], Switzerland (1/10) [11]
Boscalid	S FUNG	14	13.1	France (2/12) [48-269], Germany (5/15) [12-144], Hungary (2/7) [18-57], Italy (3/12) [13-43], Sweden (2/2) [147-1081]
Thiacloprid	SNEO	14	13.1	Austria (1/3) [24], Germany (8/15) [18-250], Hungary (3/7) [22-33], Poland (1/7) [147], Switzerland (1/10) [31]
Dimethomorph	S FUNG	11	10.3	Italy (11/12) [20-2045]
Fenhexamid	FUNG SBI	9	8.4	Germany (1/15) [2550], Hungary (1/7) [13], Italy (6/12) [11-43], Romania (1/10) [13]
Folpet	FUNG	9	8.4	France (1/12) [11], Hungary (1/7) [97], Italy (6/12) [10-1316], Romania (1/10) [51]
Trifloxystrobin	PS FUNG	9	8.4	Germany (2/15) [26-1104], Italy (7/12) [22-220]
Spiroxamine	FUNG SBI	8	7.5	Germany (1/14) [10], Italy (7/12) [12-83]
Thiophanate-methyl	S FUNG	8	7.5	France (1/12) [24], Germany (1/15) [17], Italy (1/12) [29], Poland (2/7) [10-68], Romania (2/10) [27-93], Switzerland (1/10) [21]
Amitraz (incl. Metabolites)	FORM	7	6.5	Germany (1/15) [11], Greece (2/10) [20-33], Hungary (4/7) [13-46]
Cyprodinil	SFUNG	7	6.5	France (1/12) [76], Germany (2/15) [454-590], Italy (2/12) [22-146], Switzerland (2/10) [91-10169]
Iprovalicarb	S FUNG	7	6.5	Italy (7/12) [11-302]
tau-Fluvalinate	PYR	7	6.5	Greece (1/10) [25], Poland (1/7) [12], Romania (4/10) [12-339], Switzerland (1/10) [15]
Azoxystrobin	S FUNG	6	5.6	Germany (2/15) [30-69], Poland (3/7) [17-22], Romania (1/10) [18]
Imidacloprid	S NEO	6	5.6	Italy (2/12) [1.7-11], Spain (4/14) [7.6-148.5]
Metalaxyl/Metalaxyl-M	S FUNG	6	5.6	Italy (6/12) [12-454]
Tebuconazole	FUNG SBI	6	5.6	Austria (1/3) [30], France (1/12) [159], Italy (3/12) [22-296], Poland (1/7) [16]
Acetamiprid	S NEO	5	4.7	Italy (1/12) [16], Poland (3/7) [17-45], Spain (1/14) [52]
Carbendazim	S FUNG	5	4.7	Germany (1/15) [10], Poland (3/7) [42-76], Romania (1/10) [99]
Fludioxonil	FUNG	5	4.7	France (1/12) [40], Germany (2/15) [119-1130], Greece (1/10) [27]
Bupirimate	FUNG	4	3.7	Italy (3/12) [10-70], Spain (1/14) [14]
Difenoconazole	FUNG SBI	3	2.8	Italy (2/12) [55-70], Switzerland (1/10) [11]
Dimoxystrobin	FUNG	3	2.8	Germany (1/15) [30], Hungary (2/7) [33-106]
Myclobutanil	FUNG SBI	3	2.8	Italy (1/12) [16], Spain (2/14) [27-41]
Phosmet	OP	3	2.8	Italy (2/12) [28-298], Spain (1/14) [44]
				2 () f () f ,

Class/Type:

CAR = carbamate, FORM = formamidine, FUNG = fungicide, HERB = herbicide, INS = misc. insecticide, NEO = neonicotinoid, OC = organochlorine, OP = organophosphate, PS = partial systemic, PYR = pyrethroid, REP = insect repellent, S = systemic, SBI = Steroid Biosynthesis Inhibitor, SYN = synergist

Pesticide	Class/Type	Frequenc detection	-	Countries in which found (Number of samples) [Concentration range in µg/kg]
		Samples	% samples	
Piperonyl butoxide (synergist)	SYN	3	2.8	Greece (1/10) [21], Romania (1/10) [103], Spain (1/14) [12]
Pirimicarb	CAR	3	2.8	France (2/12) [20-21], Switzerland (1/10) [16]
Quinoxyfen	FUNG	3	2.8	Italy (3/12) [19-25]
Terbuthylazin	HERB	3	2.8	Germany (1/15) [13], Italy (1/12) [22], Poland (1/7) [12]
Buprofezin	INS	2	1.9	Italy (2/12) [20-25]
Clothianidin	SNEO	2	1.9	Austria (1/3) [4.7], Sweden (1/2) [1.8]
Coumaphos	OP	2	1.9	Greece (1/10) [35], Spain (1/14) [23]
Flusilazole	OS	2	1.9	Poland (1/7) [34], Switzerland (1/10) [973]
Kresoxim-methyl	SFUNG	2	1.9	Italy (1/12) [24], Sweden (1/2) [28]
Penconazole	FUNG SBI	2	1.9	Italy (2/12) [13-102]
Pendimethalin	HERB	2	1.9	Austria (1/3) [10], Germany (1/15) [24]
Pyrimethanil	FUNG	2	1.9	Italy (1/12) [16], Switzerland (1/10) [169]
Chlorpyrifos (-methyl)	OP	1	0.9	Italy (1/12) [20]
DDE (Sum)	OC	1	0.9	Spain (1/14) [15]
DEET	REP	1	0.9	Switzerland (1/10) [28]
Dimethoate	OP	1	0.9	Spain (1/14) [26]
Dodin	FUNG	1	0.9	France (1/12) [39]
Epoxiconazole	FUNG SBI	1	0.9	Romania (1/10) [66]
Famoxadone	FUNG	1	0.9	Greece (1/10) [30]
Fenpropimorph	FUNG	1	0.9	Germany (1/15) [42]
Flufenoxuron	INS	1	0.9	Italy (1/12) [10]
HCH-Isomers (except gamma-HCH)	OC	1	0.9	Romania (1/10) [13]
Indoxacarb	INS	1	0.9	Spain (1/14) [25]
Lindane (gamma-HCH)	OC	1	0.9	Romania (1/10) [16]
Methiocarb	CAR	1	0.9	Spain (1/12) [21]
Permethrin (Sum of all Isomers)	PYR	1	0.9	Romania (1/10) [35]
Phosalone	OP	1	0.9	Switzerland (1/10) [12]
Pyraclostrobin	FUNG	1	0.9	Germany (1/15) [32]
Spinosad	INS	1	0.9	Spain (1/12) [13]
Tolylfluanid	FUNG	1	0.9	Switzerland (1/10) [44]

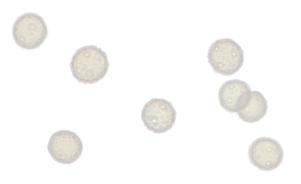
Table 2: Showing class and type of all detected pesticides and related compounds in trapped bee-foraged pollen, ordered according to frequency of detection and including the number and percentage of samples in which they were found, together with the country of origin. The overall concentration or concentration range reported is also recorded in square brackets in column 5.

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Country	Sampling period in 2013	Number of samples	Pesticides (Number of samples in which found) [Concentration range in µg/kg]
Austria	May & Sep	5	tau-Fluvalinate (1) [76], DEET (1) [17]
France	Mar	3	Amitraz (1) [503], Dimethomorph (1) [37], Pentachloroanisole* (1) [10], Folpet (1) [92], tau-Fluvalinate (1) [93]
Germany	Mar & Jun	3	Fludioxonil (1) [17], Cyprodinil (1) [18], Fenhexamid (1) [13]
Hungary	Apr	3	tau-Fluvalinate (1) [98], Coumaphos (1) [148], Carbendazim (1) [14], Tebuconazole (1) [27]
Poland	Mar-Apr	3	Fludioxonil (1) [129], Cyprodinil (1) [64], Amitraz (1) [137], Boscalid (1) [12], Chlorpyrifos (-ethyl) (1) [13]
Spain	Mar-Apr	3	Chlorpyrifos (-ethyl) (1) [99], tau-Fluvalinate (3) [11-13], Coumaphos (2) [204-12073], Carbendazim (1) [153], Pirimicarb (1) [16], Buprofezin (1) [10], Propargite (1) [26], Acrinathrin (1) [22]
Switzerland	Apr	5	Amitraz (4) [31-177]

^{*}degradation product of pentachlorophenol or pentachloronitrobenzene

Table 3: Pesticides found in samples of comb pollen/beebread, indicating the numbers of samples in which they were detected and concentration ranges reported, for each country from which samples were obtained. The sampling period within which samples were obtained is shown in column 2. Most of these samples were of beebread from the 2012 foraging season and stored over the 2012-2013 winter period, except for some samples from Austria and Germany which were obtained during the 2013 foraging season.



Pesticide	Class/ Type	Frequence	•	Countries in which found (Number of samples) [Concentration range in μ g/kg]
		Samples	% samples	
Amitraz	FORM	6	24	France (1) [503], Poland (1) [137], Switzerland (4) [31-177]
tau-Fluvalinate	PYR	6	24	Austria (1) [76], France (1) [93], Hungary (1) [98], Spain (3) [11-13]
Coumaphos	OP	3	12	Hungary (1) [148], Spain (2) [204-12073]
Carbendazim	S FUNG	2	8	Hungary (1) [14], Spain (1) [153]
Chlorpyrifos (-ethyl)	OP	2	8	Poland (1) [13], Spain (1) [99]
Cyprodinil	SFUNG	2	8	Germany (1) [18], Poland (1) [64]
Fludioxonil	FUNG	2	8	Germany (1) [17], Poland (1) [129]
Acrinathrin	PYR	1	4	Spain (1) [22]
Boscalid	SFUNG	1	4	Poland (1) [12]
Buprofezin	INS	1	4	Spain (1) [10]
DEET	REP	1	4	Austria (1) [17]
Dimethomorph	SFUNG	1	4	France (1) [37]
Fenhexamid	FUNG	1	4	Germany (1) [13]
Folpet	FUNG	1	4	France (1) [92]
Pentachloroanisole	DEG*	1	4	France (1) [10]
Pirimicarb	CAR	1	4	Spain (1) [16]
Propargite	MITI	1	4	Spain (1) [26]
Tebuconazole	FUNG	1	4	Hungary (1) [27]

^{*}degradation product of pentachlorophenol or pentachloronitrobenzene

Class/Type:

CAR = carbamate, DEG = probable degradation product of other active ingredients, FORM = formamidine, FUNG = fungicide, INS = misc. insecticide, MITI = miticide (acaricide), OP = organophosphate, PYR = pyrethroid, REP = insect repellent, S = systemic

Table 4: Showing class and type of all detected pesticides and related compounds in comb pollen/beebread, ordered according to frequency of detection and including the number and percentage of samples in which they were found, together with the country of origin. The overall concentration or concentration range reported is also recorded in square brackets in column 5.



DISCUSSION



Bees making a queen cup, in order to raise a queen.

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The range of chemicals found, particularly in trapped pollen samples, suggests that bees may be exposed to complex mixtures of agrochemicals while foraging, and that these agrochemicals are transported back to the hive and may be stored in food reserves (beebread). It is probable that exposure to specific chemicals may vary over the foraging season, as crops mature and successive chemical treatments are applied. The sampling programme and timing of sampling in the current study provide only a "snapshot" of residues present over the 12 European countries within a somewhat restricted time window. It is also not possible to use the results to represent or to compare directly the contamination status of individual countries, since samples were collected from a wide variety of locations with different cropping profiles, and at locally different phases of the cropping cycle. Nevertheless, this study has carried out simultaneous sampling in more countries than any previous study, and covers a very wide geographical area.

Even with the above limitations in mind, the tabulated information for trapped pollen indicates widespread use of chlorpyrifos – a bee-harming organophosphate insecticide and candidate for priority phase-out – and of the neonicotinoid insecticide thiacloprid, as well as the fungicide boscalid. The data also point towards more localised (or perhaps locally more intensive) use of certain chemicals, such as dimethomorph in Italy. The overall variety of fungicides present in Italian samples is also striking, pointing towards intensive local use of these active ingredients in grape growing. Intriguingly, one sample from Spain points to the historic use of DDT, while another suggests the use of technical-HCH with its mixture of HCH isomers.

Accordingly, it appears that bee-foraged pollen collected in traps is an extremely useful matrix for analysis of pesticide residues to which bees have been exposed in the course of their foraging, and which they have then carried back to the hive. In order to gain a full understanding of pesticide dynamics over the whole growing season, it is clear that a much more intense sampling campaign over the whole foraging season would need to be carried out.

THE BEES' BURDEN

AN ANALYSIS OF PESTICIDE RESIDUES IN COMB POLLEN (BEEBREAD) AND TRAPPED POLLEN FROM HONEY BEES (APIS MELLIFERA)
IN 12 EUROPEAN COUNTRIES





Some of the pesticides detected have probably been introduced into the hive primarily as a result of their use in parasite (*Varroa*) control. Amitraz, tau-fluvalinate and coumaphos fall into this category, and this may account for their relatively frequent occurrence in the samples of comb pollen (beebread). Amitraz (including its metabolites), however, was also detected in seven samples of trapped pollen and – given its potential use on a wide variety of field crops (in addition to its use as an ectoparasiticide for bees) – may well have originated in part from field sources. Tau-fluvalinate was present in seven trapped pollen samples, but also has some in-field applications on plants, such that bees could also have been exposed to plant pollen contaminated with this substance through its use outside the hive. Coumaphos was present in two samples of trapped pollen. This appears to be used only as an ectoparasiticide, though on a variety of farm animals as well as on bees.

The in-hive use of amitraz, tau-fluvalinate and coumaphos is suggested by the relatively high frequency of detection in comb pollen/beebread samples obtained for this study (24%, 24% and 12% of samples respectively). As noted above, these samples were of overwintered pollen foraged by bees in the 2012 growing season. It seems possible, therefore, that the relatively low overall numbers of pesticides detected compared to those in fresh pollen reflect a lowered use of agrochemicals in the fields at the end of the crop growing season. In addition, it is possible that the storage of the pollen in the comb over the winter, coupled with microbiological processes (lactic acid fermentation) taking place in the cells (Campos *et al* 2010), may have resulted in degradation of some pesticides originally present down to levels below detection limits.

The three restricted neonicotinoid pesticides were present in relatively few of the samples in the current study. Imidacloprid was present in 5.6% of the pollen samples, while clothianidin was present in only 1.8% (two of 107 samples). Mullin et al (2010) reported imidacloprid (all forms) present in 3.5% of their 350 samples from the US, and thiamethoxam in only 0.3%. In the data set reported by Stoner & Eitzer (2013), also for the US, imidacloprid (all forms) was detected in 12.4% of the 313 samples analysed, and thiamethoxam in 1% of samples. The data presented by Genersch et al (2010) for Germany show that imidacloprid was detected in 0.47% of the samples of pollen collected in 2007, but not in those collected during 2005/2006. Imidacloprid was found in 8.4% of samples of honey taken by Nguyen et al (2009), but always at levels below the limit of quantification for the method used. By contrast, imidacloprid (including 6-chloronicotinic acid) was found in 57.3% of samples collected in a three-year survey by Chauzat et al (2009). The high detection rates reported for France in the Chauzat et al (2009) study were not confirmed in work carried out by Lambert et al (2013) in Western France, where only 0.8% of pollen samples tested positive for imidacloprid. Bernal et al (2010) did not report any of the restricted neonicotinoids to be present in autumn- or spring-sampled stored bee pollen from Spain. Hence, the relatively low numbers of samples found in the current study to be contaminated with the three neonicotinoids banned for some uses in Europe are broadly consistent with data reported in the existing literature (see also Blacquiere et al 2012). It is likely that much depends upon how great a proportion of crops planted in the regions studied used neonicotinoid seed treatments, together with the proximity of sampled hives to such crops. In order to assess the effectiveness of the current restrictions on neonictoninoids, there is a need to carry out systematic surveillance monitoring. Only in this way will it be possible to assess any changes in releases to the environment and in exposure to bees. Currently no such programme appears to be in place.

The data reported here, in common with many of the studies cited in this report, have provided further evidence that bees are exposed to a potentially wide spectrum of pesticides, and that these pesticides can be carried back to the hive by foraging worker bees as contaminants in pollen. Conventional toxicity evaluations are based upon the premise that most mixtures of chemicals exhibit toxicity based upon a simple additivity (Concentration Addition or CA) model or an Independent Action (IA) model, with either model being applicable in many cases and generating similar (though not identical) results (see Cedergreen et al 2012, Hadrup et al 2013, Spurgeon et al 2010). It is recognised, however, that neither model predicts adequately the toxicity of some mixtures where the effect is more than additive (synergistic), and this has led for example to the development of a Generalised Addition Concentration (GCA) model from the CA model, which has proven more successful in predicting the behaviour of some groups of chemicals which interact in a synergistic fashion.

There is growing evidence that components of the mixtures of chemicals found in this and in other studies may be capable of interacting in just such a synergistic manner. In particular, some fungicides that have generally been considered as relatively safe for bees have proven to be harmful in the presence of other pesticides. For example, Norgaard & Cedergreen (2010) reported such interactions between fungicides and insecticides in experiments using aquatic test organisms. Johnson *et al* (2013) have systematically investigated the interactions between chemicals used deliberately in hives (acaricides and antimicrobials) with some agrochemicals that bees might be exposed to while foraging and through consumption of collected pollen and nectar. It was found that some commonly used acaricides interacted variously with other substances.

The toxicity of tau-fluvalinate, in particular, was increased when exposure took place in combination with 15 of the 17 other compounds tested, although such interactions were only observed for one of the 15 compounds tested with amitraz, while the other acaricides tested showed intermediate numbers of interactions.

Significantly, fungicides from the sterol biosynthesis inhibitor (SBI) class seemed to produce the greatest synergistic effects, with prochloraz causing a 2,000-fold increase in the toxicity of tau-fluvalinate in one experiment, and with other SBI fungicides also acting synergistically. Counter-intuitively, low doses of some fungicides combined with tau-fluvalinate acted in an antagonistic (less than additive) manner. While noting the potential complexity and spectrum of variability of interactions, Johnson *et al* (2013) postulated that pollen contaminated with mixtures of pesticide residues could be of toxicological significance.

Much earlier studies also pointed towards synergistic interactions. For example, Vandame et al (1995) found that exposure to deltamethrin in combination with the fungicides prochloraz or difenoconazole induced hypothermia in honey bees at doses that did not induce a significant effect on thermoregulation when used alone. Iwasa et al (2004) showed that the neonicotinoids thiacloprid and acetamiprid were more toxic to bees with simultaneous exposure to fungicides, even though these insecticides are considered not to be highly toxic to bees as individual active agents. The toxicity to honey bees of acetamiprid increased by a factor of 244 when present in combination with triflumizole, and by 105 times with propiconazole. The toxicity of thiacloprid to bees increased even more dramatically in the same combinations, by factors of 1,141 and 559 respectively.







In 2012, a report published by the European Food Safety Authority (EFSA) stated:

"Significant synergy has been reported between EBI [SBI] fungicides and both neonicotinoids and pyrethroid insecticides but in some cases where high levels of synergy are reported the doses of fungicides have been well in excess of those identified in the exposure section of this report. ... Greater synergy is observed in the laboratory between EBI fungicides at field rates application rates [sic] and pyrethroids used as varroacides (flumethrin and fluvalinate) and between coumaphos and fluvalinate varroacides." (see Thompson 2012).

Gill et al (2012) constructed a field-realistic scenario in which bumblebees received long-term (four-week) exposure to two common insecticides, imidacloprid (neonicotinoid) and lambda-cyhalothrin (pyrethroid), at field-relevant doses. Natural foraging behaviours were impaired and worker mortality increased, which lead to reductions in brood development. The combined effect of the two insecticides was greater than that observed with separate exposure, leading the authors to consider that the results provided "evidence that combinatorial exposure to pesticides increases the propensity of colonies to fail". More recently still, Zhu et al (2014) reported complex interactions of acaricides and insecticides, with a concentration-dependent switch from synergism to antagonism observed in a binary mixture of chlorothalonil and tau-fluvalinate. In that study, it was also found that the common pesticide additive N-methyl-2-pyrrolidone (NMP) was highly toxic to honey bee larvae, although it is considered in an inert agent when used as a component of formulations.

There is also evidence that exposure to pesticides may increase the susceptibility of bees to infections with the gut parasite *Nosema ceranae*. Alaux *et al* (2010) showed that the combined effects of imidacloprid and *Nosema* infestation significantly weakened honey bees, causing high mortality and high levels of stress and ultimately weakening the colony as a whole. In another study, a proportion of bees reared from brood comb with high levels of pesticide residues were found to become infected with *Nosema ceranae* at a younger age, compared to those reared in low-residue brood combs (Wu *et al* 2012). Pettis *et al* (2013) found that two fungicides (chlorothalonil and pyraclostrobin) and two miticides used in-hive (fluvalinate and amitraz) increased honey bee susceptibility to infection with the parasite. Another recent study has shown that exposure to sub-lethal doses of the pesticides fipronil and thiacloprid caused much higher mortality to honey bees previously infected with *N. ceranae* than in those with no history of infection (Vidau *et al* 2011).

Di Prisco *et al* (2013) have shown that clothianidin and imidacloprid can compromise aspects of the immune system of bees, which in turn allows Deformed Wing Virus (DWV) to replicate, and covert viral infections to be expressed as overt disease. DWV also has a complex relationship with *Varroa* infestations.

In summary, the work reported in this study confirms and is consistent with the findings of other studies that have shown trapped bee pollen and comb pollen/beebread to be contaminated with a wide variety of pesticides, using samples drawn from a wider geographical range of locations across Europe than has previously been sampled. Some of the pesticides identified have been shown in other studies to act together synergistically and also to act singly or in combination to increase the susceptibility of bees to diseases and parasites.



Bees and other pollinators undoubtedly face many pressures in the contemporary agricultural environment, with threats from habitat and biodiversity loss, spread of disease and parasites and changing climatic conditions acting alongside, and perhaps in concert with, the threat from multiple exposure to pesticide residues in pollen, nectar and guttation fluid. Strategies to protect bees and other insects on which agriculture and horticulture relies, and which form a vital component of natural ecosystems, must therefore be capable of addressing a diversity of stressors.

Although no one measure alone is likely to be sufficient, it is possible to identify key policy and practical actions that could begin to reverse the decline and secure the long-term future of these species:

- In order to confer greater protection to wild and cultured pollinators, restrictions
 on the use of the systemic insecticides imidacloprid, thiamethoxam, clothianidin
 and fipronil in seed dressings, soil applications and foliar spraying should be made
 permanent, and expanded in scope to include other uses.
- 2. Furthermore, other insecticides known to be very harmful to bees, including chlorpyrifos and the synthetic pyrethoids cypermethrin and deltamethrin, should also be brought under strict control.
- Surveillance monitoring of pesticides to which pollinators are exposed needs to address the widest possible spectrum of substances (and their metabolites) which can be resolved using state-of-the-art analytical methods at the best achievable detection limits.
- 4. The fact that pollinators are commonly exposed to pesticides as mixtures needs to be taken fully into account, particularly the possibility of synergistic interactions, recognising that these may remain difficult to predict quantitatively using many of the currently available models of joint toxicity. Accordingly, strategies should also be developed which aim at, and result in, a substantial overall reduction in use of pesticides of all types as a precautionary goal in itself.
- 5. Coordinated Bee Action Plans need to be established, which aim not only at more effective regulation and control of agricultural chemicals, but also facilitate the monitoring of the health of bees and other pollinators. They should also work to improve the conservation of natural and semi-natural habitats around agricultural landscapes, as well as enhancing biodiversity within agricultural fields.
- 6. Funding for research and development of ecological farming practices should be radically increased, in order to catalyse a move away from reliance on chemical pest control towards biodiversity-based tools to control pests and enhance ecosystem health. EU policy makers should direct more funding for ecological agriculture solutions research under the auspices of the CAP (direct payments) and Horizon 2020 (EU research) programmes.

APPENDIX 1: FULL DATA

Country	Date sampled	Location		Neonicotino	oid insecticides (µ	g/kg pollen)	
			Imidacloprid	Clothianidin	Thiamethoxam	Thiacloprid	Acetamiprid
Austria	09.05.2013	Styria, Fürstenfeld		4.7			
	09.05.2013	Styria, Fürstenfeld					
	May 2013	Grieskirchen, Rottenbach, Upper Austria				24	
France	14.04.2013	Rhöne-Alpes SE France (near Lyon)					
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)					
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)					
	19.06.2013	Ain-East Center France, north from the Alps					
	26.06.2013	Ain-East Center France, north from the Alps					
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"					
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse					
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse					
	01.08.2013	Pyrenees, S France					
	28.08.2013	Midi-Pyrénais, SW France, near Toulouse					
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"					
	End of July	Ain-East Center France, north from the Alps					
Germany	22.05.2013	Rheinberg				76	
	11.06.2013	21521 Dassendorf				250	
	30/31.05.2013	21465 Wentorf				18	
	15.05.2013	Sachsen, OT Großbardan, botanical reserve Totenberg					
	28.05.2013	23847 Pölitz				85	
	20.05.2013	Baden-Württemberg, 88521 Ertingen				53	
	15/16.05.2013	Lower Saxony, 31241 Kleine Ilsede					
	22.05.2013	Baden Württemberg, 68809 Neulaßheim				106	
	29.05.2013	Baden-Württemberg, 71717 Beilstein				220	
	19.05.2013	Nordrhein Westfalen, 48324 Sendenhorst					
	21.05.2013	Saxony, 09123 Chemnitz				10	
	02.06.2013	Baden Württemberg, 72348 Rosenfeld					
	15.05.2013	Baden Württemberg, 74626 Bretzfeld					
	15/16.05.2013	Saxony, 01683 Nossen					
	06.06.2013	Schleswig Holstein, 25899 Herrenkrog					

Table A1:

Country	Date sampled	Location		Neonicotino	oid insecticides (μ	g/kg pollen)	
			Imidacloprid	Clothianidin	Thiamethoxam	Thiacloprid	Acetamiprid
Greece	27.06.2013	Trikala / Thessalia					
	28.06.2013	Voiotia					
	28.06.2013	Voiotia					
	01.07.2013	Arta/ Epiros					
	28.06.2013	Halkidiki					
	28.06.2013	Halkidiki					
	16.07.2013	Soufli, Evros					
	28.06.2013	Redestos, Thessaloniki					
	28.06.2013	Redestos, Thessaloniki					
	19.07.2013	Lagadas, Thessaloniki					
Hungary	28.05.2013	Kisbodak				30	
	10.07.2013	Siófok				22	
	28.05.2013	Nagykónyi					
	10.07.2013	Nagykónyi					
	28.05.2013	Siófok				33	
	10.07.2013	Egyházaskozàr					
	10.07.2013	Tab					
Italy	15.05.2013	Ponte in Valtellina - Loc. S.Rocco	11				16
	02.06.2013	Corte Franca (Brescia)					
	07.06.2013	Corte Franca (Brescia)					
	13.06.2013	Corte Franca (Brescia)					
	19.06.2013	Corte Franca (Brescia)					
	23.06.2013	Montebelluna - via Bonulovanni 92					
	26.06.2013	Montebelluna					
	27.06.2013	Montebelluna					
	16.06.2013	Cisterna d'Asti, Valle San Matteo	1.7				
	23.06.2013	Cisterna d'Asti, Valle San Matteo					
	30.06.2013	Cisterna d'Asti, Valle San Matteo					
	07.07.2013	Cisterna d'Asti, Valle San Matteo					
Luxembourg	04.06.2013	Municipality of Biwer, E Lux					
	01.06.2013	Municipality of Manternach					
	03.06.2013	Municipality of Clemency					
	28.05.2013	Municipality of Clemency					
	04.06.2013	Municipality of Linger					

Table A1 (continued):

Country	Date sampled	Location		Neonicotino	oid insecticides (µ	g/kg pollen)	
	•		Imidacloprid	Clothianidin	Thiamethoxam	Thiacloprid	Acetamiprid
Poland	28.05.2013	GA,Siorowo, Oziatdowo County Warminsko-Mazurskie					45
	28.05.2013	Jankowice, Dziatdowo county, Warminsko					17
	14.06.2013	Miedzna slaskie					29
	12.06.2013	Sinki				147	
	16.06.2013	Stawiec					
	28.05.2013	Matujowice					
	30.05.2013	Matujowice Koto Brzegu					
Romania	16.06.2013	Daia Română (Alba County)					
	25.06.2013	Hârşeşti (Argeş County)					
	26.06.2013	Pașcani (lași County)					
	28.06.2013	Şuştra (Timiş County)					
	29.07.2013	Drogu-Udrești (Brăila County)					
	30.07.2013	Cilibia (Buzău County)					
	02.08.2013	Făcăieni (lalomița County)					
	07.08.2013	Gostinu (Giurgiu County)					
	07.08.2013	Badeana (Vaslui County)					
	20.08.2013	Moara Vlasiei (Ilfov County)					
Spain	28.07.2013	La Santa Espina (Castromonte) en Valladolid					
	28.07.2013	La Santa Espina (Castromonte) en Valladolid					
	20-21.08.2013	Hombrados (Guadalajara)					
	30.07.2013	Argamasilla de Alba (Ciudad Real)	7.6				
	30.07.2013	Argamasilla de Alba (Ciudad Real)	17.9				
	30.07.2013	Llanos de Caudillo (Ciudad Real)	30.4				
	29.07.2013	Cádiz					
	12.08.2013	Alcázar del Rey (Cuenca)					
	13.08.2013	Bocairent (Valencia)					
	05.08.2013	Cabezas de San Juan (Sevilla)	148.5				
	17.07.2013	Córdoba (no entiendo el municipio)					
	19.08.2013	Montoro (Sevilla)					52
	08.08.2013	Tarazona de la Guareña (Salamanca)					
	25.08.2013	Plana de Alcañiz (Teruel)					
Sweden	05.07.2013	Vikbolandet					
	03.07.2013	Vikbolandet		1.8			

Table A1 (continued):

Country	Date sampled	Location		Neonicotino	oid insecticides (µ	g/kg pollen)	
			Imidacloprid	Clothianidin	Thiamethoxam	Thiacloprid	Acetamiprid
Switzerland	06.05.2013	Laupersdorf				31	
	08.05.2013	Ogens					
	13.05.2013	Ecublens					
	07.05.2013	Brent					
	21.05.2013	Wabern bei Bern					
	23.05.2013	Melchnau					
	01.06.2013	Melchnau					
	18.08.2013	Laupersdorf					
	15.04.2013	Attiswil					
	02.09.2013	Attiswil					

Table A1 (continued):

Country	Date sampled	Location			Other	insectic	ides/aca	ıricides (ug/kg pc	llen)*		
			A & M	Bup.	Ch(-e)	Ch(-m)	Cou.	Flu.	tau-F	Ph'l.	Ph'm.	Pir.
Austria	09.05.2013	Styria, Fürstenfeld										
	09.05.2013	Styria, Fürstenfeld										
	May 2013	Grieskirchen, Rottenbach, Upper Austria										
France	14.04.2013	Rhöne-Alpes SE France (near Lyon)										
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)										20
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)										21
	19.06.2013	Ain-East Center France, north from the Alps										
	26.06.2013	Ain-East Center France, north from the Alps										
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"										
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse										
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse										
	01.08.2013	Pyrenees, S France										
	28.08.2013	Midi-Pyrénais, SW France, near Toulouse			10							
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"										
	End of July	Ain-East Center France, north from the Alps										
Germany	22.05.2013	Rheinberg										
	11.06.2013	21521 Dassendorf										
	30/31.05.2013	21465 Wentorf										
	15.05.2013	Sachsen, OT Großbardan, botanical reserve Totenberg										
	28.05.2013	23847 Pölitz	11									
	20.05.2013	Baden-Württemberg, 88521 Ertingen										
	15/16.05.2013	Lower Saxony, 31241 Kleine Ilsede										
	22.05.2013	Baden Württemberg, 68809 Neulaßheim										
	29.05.2013	Baden-Württemberg, 71717 Beilstein										
	19.05.2013	Nordrhein Westfalen, 48324 Sendenhorst										
	21.05.2013	Saxony, 09123 Chemnitz										
	02.06.2013	Baden Württemberg, 72348 Rosenfeld										
	15.05.2013	Baden Württemberg, 74626 Bretzfeld										
	15/16.05.2013	Saxony, 01683 Nossen										
	06.06.2013	Schleswig Holstein, 25899 Herrenkrog										

Table A2:

Country	Date sampled	Location			Othe	r insectici	ides/aca	ıricides (ug/kg po	llen)*		
			A & M	Bup.	Ch(-e)	Ch(-m)	Cou.	Flu.	tau-F	Ph'l.	Ph'm.	Pir.
Greece	27.06.2013	Trikala / Thessalia	20		360							
	28.06.2013	Voiotia										
	28.06.2013	Voiotia										
	01.07.2013	Arta/ Epiros	33				35		25			
	28.06.2013	Halkidiki										
	28.06.2013	Halkidiki										
	16.07.2013	Soufli, Evros										
	28.06.2013	Redestos, Thessaloniki										
	28.06.2013	Redestos, Thessaloniki										
	19.07.2013	Lagadas, Thessaloniki										
Hungary	28.05.2013	Kisbodak	46		123							
	10.07.2013	Siófok	19									
	28.05.2013	Nagykónyi										
	10.07.2013	Nagykónyi										
	28.05.2013	Siófok	35									
_	10.07.2013	Egyházaskozàr	13									
	10.07.2013	Tab										
Italy	15.05.2013	Ponte in Valtellina - Loc. S.Rocco										
	02.06.2013	Corte Franca (Brescia)										
	07.06.2013	Corte Franca (Brescia)										
	13.06.2013	Corte Franca (Brescia)										
	19.06.2013	Corte Franca (Brescia)										
	23.06.2013	Montebelluna - via Bonulovanni 92										
	26.06.2013	Montebelluna										
	27.06.2013	Montebelluna		20				10				
	16.06.2013	Cisterna d'Asti, Valle San Matteo			10						28	
	23.06.2013	Cisterna d'Asti, Valle San Matteo		25								
	30.06.2013	Cisterna d'Asti, Valle San Matteo			13							
	07.07.2013	Cisterna d'Asti, Valle San Matteo			562	20					298	
Luxembourg	04.06.2013	Municipality of Biwer, E Lux										
	01.06.2013	Municipality of Manternach										
	03.06.2013	Municipality of Clemency										
	28.05.2013	Municipality of Clemency										
	04.06.2013	Municipality of Linger										

Table A2 (continued):

Country	Date sampled	Location	Other insecticides/acaricides (µg/kg pollen)*									
			A & M	Bup.	Ch(-e)	Ch(-m)	Cou.	Flu.	tau-F	Ph'l.	Ph'm.	Pir.
Poland	28.05.2013	GA, Siorowo, Oziatdowo County Warminsko-Mazurskie			90							
	28.05.2013	Jankowice, Dziatdowo county, Warminsko			25							
	14.06.2013	Miedzna slaskie			10							
	12.06.2013	Sinki			119							
	16.06.2013	Stawiec										
	28.05.2013	Matujowice			104				12			
	30.05.2013	Matujowice Koto Brzegu			118							
Romania	16.06.2013	Daia Română (Alba County)							13			
	25.06.2013	Hârşeşti (Argeş County)							339			
	26.06.2013	Pașcani (Iași County)										
	28.06.2013	Şuştra (Timiş County)										
	29.07.2013	Drogu-Udrești (Brăila County)										
	30.07.2013	Cilibia (Buzău County)										
	02.08.2013	Făcăieni (Ialomița County)							122			
	07.08.2013	Gostinu (Giurgiu County)							12			
	07.08.2013	Badeana (Vaslui County)										
	20.08.2013	Moara Vlasiei (Ilfov County)										
Spain	28.07.2013	La Santa Espina (Castromonte) en Valladolid										
	28.07.2013	La Santa Espina (Castromonte) en Valladolid										
	20-21.08.2013	Hombrados (Guadalajara)										
	30.07.2013	Argamasilla de Alba (Ciudad Real)			11							
	30.07.2013	Argamasilla de Alba (Ciudad Real)			15							
	30.07.2013	Llanos de Caudillo (Ciudad Real)			129							
	29.07.2013	Cádiz										
	12.08.2013	Alcázar del Rey (Cuenca)										
	13.08.2013	Bocairent (Valencia)									44	
	05.08.2013	Cabezas de San Juan (Sevilla)			18							
	17.07.2013	Córdoba (no entiendo el municipio)										
	19.08.2013	Montoro (Sevilla)			705		23					
	08.08.2013	Tarazona de la Guareña (Salamanca)										
	25.08.2013	Plana de Alcañiz (Teruel)										
Sweden	05.07.2013	Vikbolandet										
	03.07.2013	Vikbolandet										

Table A2 (continued):

Country	Date sampled	Location	Other insecticides/acaricides (µg/kg pollen)*									
			A & M	Bup.	Ch(-e)	Ch(-m)	Cou.	Flu.	tau-F	Ph'l.	Ph'm.	Pir.
Switzerland	06.05.2013	Laupersdorf										
	08.05.2013	Ogens			11							
	13.05.2013	Ecublens								12		16
	07.05.2013	Brent										
	21.05.2013	Wabern bei Bern							15			
	23.05.2013	Melchnau										
	01.06.2013	Melchnau										
	18.08.2013	Laupersdorf										
	15.04.2013	Attiswil										
	02.09.2013	Attiswil										

* Key to columns:

A & M Amitraz and metabs

Bup. Buprofezin

Ch(-e) Chlorpyrifos (-ethyl) Ch(-m) Chlorpyrifos (-methyl)

Cou. Coumaphos
Flu. Flufenoxuron
tau-F tau-Fluvalinate
Ph'l. Phosalone
Ph'm. Phosmet
Pir. Pirimicarb

Table A2 (continued):

Country	Date sampled	Location	Other insecticides/acaricides (µg/kg pollen)*									
			HCH-i	Lind.	Perm.	Dim.	DDE	Meth.	Spin.	Indox.	Pendi.	Terb.
Austria	09.05.2013	Styria, Fürstenfeld									10	
	09.05.2013	Styria, Fürstenfeld										
	May 2013	Grieskirchen, Rottenbach, Upper Austria										
France	14.04.2013	Rhöne-Alpes SE France (near Lyon)										
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)										
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)										
	19.06.2013	Ain-East Center France, north from the Alps										
	26.06.2013	Ain-East Center France, north from the Alps										
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"										
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse										
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse										
	01.08.2013	Pyrenees, S France										
	28.08.2013	Midi-Pyrénais, SW France, near Toulouse										
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"										
	End of July	Ain-East Center France, north from the Alps										
Germany	22.05.2013	Rheinberg										
	11.06.2013	21521 Dassendorf										
	30/31.05.2013	21465 Wentorf										
	15.05.2013	Sachsen, OT Großbardan, botanical reserve Totenberg										
	28.05.2013	23847 Pölitz										
	20.05.2013	Baden-Württemberg, 88521 Ertingen										
	15/16.05.2013	Lower Saxony, 31241 Kleine Ilsede										
	22.05.2013	Baden Württemberg, 68809 Neulaßheim									24	
	29.05.2013	Baden-Württemberg, 71717 Beilstein										
	19.05.2013	Nordrhein Westfalen, 48324 Sendenhorst										
	21.05.2013	Saxony, 09123 Chemnitz										
	02.06.2013	Baden Württemberg, 72348 Rosenfeld										
	15.05.2013	Baden Württemberg, 74626 Bretzfeld										
	15/16.05.2013	Saxony, 01683 Nossen										
	06.06.2013	Schleswig Holstein, 25899 Herrenkrog										13

Table A3:

Country	Date sampled	Location			Other	rinsectio	cides/ac	aricides	(µg/kg p	ollen)*		
			HCH-i	Lind.	Perm.	Dim.	DDE	Meth.	Spin.	Indox.	Pendi.	Terb.
Greece	27.06.2013	Trikala / Thessalia										
	28.06.2013	Voiotia										
	28.06.2013	Voiotia										
	01.07.2013	Arta/ Epiros										
	28.06.2013	Halkidiki										
	28.06.2013	Halkidiki										
	16.07.2013	Soufli, Evros										
	28.06.2013	Redestos, Thessaloniki										
	28.06.2013	Redestos, Thessaloniki										
	19.07.2013	Lagadas, Thessaloniki										
Hungary	28.05.2013	Kisbodak										
	10.07.2013	Siófok										
	28.05.2013	Nagykónyi										
	10.07.2013	Nagykónyi										
	28.05.2013	Siófok										
	10.07.2013	Egyházaskozàr										
	10.07.2013	Tab										
Italy	15.05.2013	Ponte in Valtellina - Loc. S.Rocco										
	02.06.2013	Corte Franca (Brescia)										
	07.06.2013	Corte Franca (Brescia)										
	13.06.2013	Corte Franca (Brescia)										
	19.06.2013	Corte Franca (Brescia)										22
	23.06.2013	Montebelluna - via Bonulovanni 92										
	26.06.2013	Montebelluna										
	27.06.2013	Montebelluna										
	16.06.2013	Cisterna d'Asti, Valle San Matteo										
	23.06.2013	Cisterna d'Asti, Valle San Matteo										
	30.06.2013	Cisterna d'Asti, Valle San Matteo										
	07.07.2013	Cisterna d'Asti, Valle San Matteo										
Luxembourg	04.06.2013	Municipality of Biwer, E Lux										
	01.06.2013	Municipality of Manternach										
	03.06.2013	Municipality of Clemency										
	28.05.2013	Municipality of Clemency										
	04.06.2013	Municipality of Linger										

Table A3 (continued):

Full data for concentrations of certain other insecticides/acaricides (and herbicides in bee pollen samples collected from hive traps during 2013, showing sampling dates and locations.

Country	Date sampled	Location			Other	rinsectio	ides/ac	aricides ((μg/kg p	ollen)*		
			HCH-i	Lind.	Perm.	Dim.	DDE	Meth.	Spin.	Indox.	Pendi.	Terb.
Poland	28.05.2013	GA,Siorowo, Oziatdowo County Warminsko-Mazurskie										
	28.05.2013	Jankowice, Dziatdowo county, Warminsko										
	14.06.2013	Miedzna slaskie										
	12.06.2013	Sinki										
	16.06.2013	Stawiec										
	28.05.2013	Matujowice										12
	30.05.2013	Matujowice Koto Brzegu										
Romania	16.06.2013	Daia Română (Alba County)										
	25.06.2013	Hârşeşti (Argeş County)										
	26.06.2013	Pașcani (Iași County)										
	28.06.2013	Şuştra (Timiş County)										
	29.07.2013	Drogu-Udrești (Brăila County)										
	30.07.2013	Cilibia (Buzău County)										
	02.08.2013	Făcăieni (lalomița County)										
	07.08.2013	Gostinu (Giurgiu County)	13	16								
	07.08.2013	Badeana (Vaslui County)			35							
	20.08.2013	Moara Vlasiei (Ilfov County)										
Spain	28.07.2013	La Santa Espina (Castromonte) en Valladolid										
	28.07.2013	La Santa Espina (Castromonte) en Valladolid										
	20-21.08.2013	Hombrados (Guadalajara)										
	30.07.2013	Argamasilla de Alba (Ciudad Real)										
	30.07.2013	Argamasilla de Alba (Ciudad Real)							13			
	30.07.2013	Llanos de Caudillo (Ciudad Real)				26						
	29.07.2013	Cádiz										
	12.08.2013	Alcázar del Rey (Cuenca)										
	13.08.2013	Bocairent (Valencia)										
	05.08.2013	Cabezas de San Juan (Sevilla)								25		
	17.07.2013	Córdoba (no entiendo el municipio)										
	19.08.2013	Montoro (Sevilla)					15					
	08.08.2013	Tarazona de la Guareña (Salamanca)										
	25.08.2013	Plana de Alcañiz (Teruel)						21				
Sweden	05.07.2013	Vikbolandet										
	03.07.2013	Vikbolandet										

Table A3 (continued):

Full data for concentrations of certain other insecticides/acaricides (and herbicides in bee pollen samples collected from hive traps during 2013, showing sampling dates and locations.

Country	Date sampled	Location			Other	insectic	ides/aca	aricides (µg/kg p	ollen)*		
			HCH-i	Lind.	Perm.	Dim.	DDE	Meth.	Spin.	Indox.	Pendi.	Terb.
Switzerland	06.05.2013	Laupersdorf										
	08.05.2013	Ogens										
	13.05.2013	Ecublens										
	07.05.2013	Brent										
	21.05.2013	Wabern bei Bern										
	23.05.2013	Melchnau										
	01.06.2013	Melchnau										
	18.08.2013	Laupersdorf										
	15.04.2013	Attiswil										
	02.09.2013	Attiswil										

Terb.

HCH-i HCH-isomers Lind. Lindane Perm. Permethrin (Sum) Dim. Dimethoate DDE DDE (Sum) Meth. Methiocarb Spin. Spinosad Indox. Indoxacarb Pendi. Pendimethalin

Terbuthylazin

Table A3 (continued):

Full data for concentrations of certain other insecticides/acaricides (and herbicides in bee pollen samples collected from hive traps during 2013, showing sampling dates and locations.

Country	Date sampled	Location				Fung	gicides (µ	ug/kg pc	ollen)*			
	·		Azox.	Bosc.	Bupiri.	Carb.	Cypr.	Dif.	Dim.	D'oxy.	Dodin	Epox.
Austria	09.05.2013	Styria, Fürstenfeld										
	09.05.2013	Styria, Fürstenfeld										
	May 2013	Grieskirchen, Rottenbach, Upper Austria										
France	14.04.2013	Rhöne-Alpes SE France (near Lyon)					76				39	
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)		269								
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)										
	19.06.2013	Ain-East Center France, north from the Alps		48								
	26.06.2013	Ain-East Center France, north from the Alps										
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"										
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse										
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse										
	01.08.2013	Pyrenees, S France										
	28.08.2013	Midi-Pyrénais, SW France, near Toulouse										
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"										
	End of July	Ain-East Center France, north from the Alps										
Germany	22.05.2013	Rheinberg										
	11.06.2013	21521 Dassendorf		114			590					
	30/31.05.2013	21465 Wentorf										
	15.05.2013	Sachsen, OT Großbardan, botanical reserve Totenberg		13						30		
	28.05.2013	23847 Pölitz										
	20.05.2013	Baden-Württemberg, 88521 Ertingen		144								
	15/16.05.2013	Lower Saxony, 31241 Kleine Ilsede										
	22.05.2013	Baden Württemberg, 68809 Neulaßheim		18								
	29.05.2013	Baden-Württemberg, 71717 Beilstein	69	12			454					
	19.05.2013	Nordrhein Westfalen, 48324 Sendenhorst										
	21.05.2013	Saxony, 09123 Chemnitz										
	02.06.2013	Baden Württemberg, 72348 Rosenfeld										
	15.05.2013	Baden Württemberg, 74626 Bretzfeld										
	15/16.05.2013	Saxony, 01683 Nossen	30			10						
	06.06.2013	Schleswig Holstein, 25899 Herrenkrog										

Table A4:

Country	Date sampled	Location				Fung	gicides (µ	ıg/kg po	ollen)*			
			Azox.	Bosc.	Bupiri.	Carb.	Cypr.	Dif.	Dim.	D'oxy.	Dodin	Epox.
Greece	27.06.2013	Trikala / Thessalia										
	28.06.2013	Voiotia										
	28.06.2013	Voiotia										
	01.07.2013	Arta/ Epiros										
	28.06.2013	Halkidiki										
	28.06.2013	Halkidiki										
	16.07.2013	Soufli, Evros										
	28.06.2013	Redestos, Thessaloniki										
	28.06.2013	Redestos, Thessaloniki										
	19.07.2013	Lagadas, Thessaloniki										
Hungary	28.05.2013	Kisbodak										
	10.07.2013	Siófok										
	28.05.2013	Nagykónyi										
	10.07.2013	Nagykónyi										
_	28.05.2013	Siófok		18						33		
	10.07.2013	Egyházaskozàr										
	10.07.2013	Tab		57						106		
Italy	15.05.2013	Ponte in Valtellina - Loc. S.Rocco					22	70				
	02.06.2013	Corte Franca (Brescia)							20			
	07.06.2013	Corte Franca (Brescia)							69			
	13.06.2013	Corte Franca (Brescia)			12				143			
	19.06.2013	Corte Franca (Brescia)		43			146		183			
	23.06.2013	Montebelluna - via Bonulovanni 92							71			
	26.06.2013	Montebelluna							46			
	27.06.2013	Montebelluna		13					325			
	16.06.2013	Cisterna d'Asti, Valle San Matteo		43	70			55	2045			
	23.06.2013	Cisterna d'Asti, Valle San Matteo							76			
	30.06.2013	Cisterna d'Asti, Valle San Matteo							138			
	07.07.2013	Cisterna d'Asti, Valle San Matteo			10				110			
Luxembourg	04.06.2013	Municipality of Biwer, E Lux										
	01.06.2013	Municipality of Manternach										
	03.06.2013	Municipality of Clemency										
	28.05.2013	Municipality of Clemency										
	04.06.2013	Municipality of Linger										

Table A4 (continued):

Country	Date sampled	Location				Fung	gicides (¿	ug/kg pc	ollen)*			
			Azox.	Bosc.	Bupiri.	Carb.	Cypr.	Dif.	Dim.	D'oxy.	Dodin	Epox.
Poland	28.05.2013	GA,Siorowo, Oziatdowo County Warminsko-Mazurskie				76						
	28.05.2013	Jankowice, Dziatdowo county, Warminsko	17			42						
	14.06.2013	Miedzna slaskie	22									
	12.06.2013	Sinki				61						
	16.06.2013	Stawiec	19									
	28.05.2013	Matujowice										
	30.05.2013	Matujowice Koto Brzegu										
Romania	16.06.2013	Daia Română (Alba County)										
	25.06.2013	Hârşeşti (Argeş County)										
	26.06.2013	Pașcani (Iași County)				99						66
	28.06.2013	Şuştra (Timiş County)										
	29.07.2013	Drogu-Udrești (Brăila County)										
	30.07.2013	Cilibia (Buzău County)										
	02.08.2013	Făcăieni (Ialomița County)	18									
	07.08.2013	Gostinu (Giurgiu County)										
	07.08.2013	Badeana (Vaslui County)										
	20.08.2013	Moara Vlasiei (Ilfov County)										
Spain	28.07.2013	La Santa Espina (Castromonte) en Valladolid										
	28.07.2013	La Santa Espina (Castromonte) en Valladolid										
	20-21.08.2013	Hombrados (Guadalajara)										
	30.07.2013	Argamasilla de Alba (Ciudad Real)										
	30.07.2013	Argamasilla de Alba (Ciudad Real)										
	30.07.2013	Llanos de Caudillo (Ciudad Real)			14							
	29.07.2013	Cádiz										
	12.08.2013	Alcázar del Rey (Cuenca)										
	13.08.2013	Bocairent (Valencia)										
	05.08.2013	Cabezas de San Juan (Sevilla)										
	17.07.2013	Córdoba (no entiendo el municipio)										
	19.08.2013	Montoro (Sevilla)										
	08.08.2013	Tarazona de la Guareña (Salamanca)										
	25.08.2013	Plana de Alcañiz (Teruel)										
Sweden	05.07.2013	Vikbolandet		147								
	03.07.2013	Vikbolandet		1081								

Table A4 (continued):

Country	Date sampled	Location				Fung	gicides (µ	/g/kg po	llen)*			
			Azox.	Bosc.	Bupiri.	Carb.	Cypr.	Dif.	Dim.	D'oxy.	Dodin	Epox.
Switzerland	06.05.2013	Laupersdorf										
	08.05.2013	Ogens										
	13.05.2013	Ecublens					91	11				
	07.05.2013	Brent					10169					
	21.05.2013	Wabern bei Bern										
	23.05.2013	Melchnau										
	01.06.2013	Melchnau										
	18.08.2013	Laupersdorf										
	15.04.2013	Attiswil										
	02.09.2013	Attiswil										

Azoxystrobin Azox. Bosc. Boscalid Bupiri. Bupirimate Carb. Carbendazim Cypr. Cyprodinil Difenoconazole Dif. Dim. Dimethomorph D'oxy. Dimoxystrobin Dodin Dodin Epox. Epoxiconazole

Table A4 (continued):

Country	Date sampled	Location				Fung	jicides (ug/kg po	ollen)*			
	·		Fam.	F'hex.	F'pro.	F'diox.	F'sil.	Folpet	lprov.	K-met	M/M	Myclo.
Austria	09.05.2013	Styria, Fürstenfeld										
	09.05.2013	Styria, Fürstenfeld										
	May 2013	Grieskirchen, Rottenbach, Upper Austria										
France	14.04.2013	Rhöne-Alpes SE France (near Lyon)				40						
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)										
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)										
	19.06.2013	Ain-East Center France, north from the Alps										
	26.06.2013	Ain-East Center France, north from the Alps						11				
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"										
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse										
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse										
	01.08.2013	Pyrenees, S France										
	28.08.2013	Midi-Pyrénais, SW France, near Toulouse										
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"										
	End of July	Ain-East Center France, north from the Alps										
Germany	22.05.2013	Rheinberg										
	11.06.2013	21521 Dassendorf		2550		1130						
	30/31.05.2013	21465 Wentorf										
	15.05.2013	Sachsen, OT Großbardan, botanical reserve Totenberg										
	28.05.2013	23847 Pölitz										
	20.05.2013	Baden-Württemberg, 88521 Ertingen										
	15/16.05.2013	Lower Saxony, 31241 Kleine Ilsede										
	22.05.2013	Baden Württemberg, 68809 Neulaßheim										
	29.05.2013	Baden-Württemberg, 71717 Beilstein				119						
	19.05.2013	Nordrhein Westfalen, 48324 Sendenhorst			42							
	21.05.2013	Saxony, 09123 Chemnitz										
	02.06.2013	Baden Württemberg, 72348 Rosenfeld										
	15.05.2013	Baden Württemberg, 74626 Bretzfeld										
	15/16.05.2013	Saxony, 01683 Nossen										
	06.06.2013	Schleswig Holstein, 25899 Herrenkrog										

Table A5:

Country	Date sampled	Location				Fung	jicides (ug/kg po	llen)*			
			Fam.	F'hex.	F'pro.	F'diox.	F'sil.	Folpet	lprov.	K-met	M/M	Myclo.
Greece	27.06.2013	Trikala / Thessalia	30			27						
	28.06.2013	Voiotia										
	28.06.2013	Voiotia										
	01.07.2013	Arta/ Epiros										
	28.06.2013	Halkidiki										
	28.06.2013	Halkidiki										
	16.07.2013	Soufli, Evros										
	28.06.2013	Redestos, Thessaloniki										
	28.06.2013	Redestos, Thessaloniki										
	19.07.2013	Lagadas, Thessaloniki										
Hungary	28.05.2013	Kisbodak										
	10.07.2013	Siófok						97				
	28.05.2013	Nagykónyi		13								
	10.07.2013	Nagykónyi										
	28.05.2013	Siófok										
	10.07.2013	Egyházaskozàr										
	10.07.2013	Tab										
Italy	15.05.2013	Ponte in Valtellina - Loc. S.Rocco										
	02.06.2013	Corte Franca (Brescia)										
	07.06.2013	Corte Franca (Brescia)										
	13.06.2013	Corte Franca (Brescia)							188			
	19.06.2013	Corte Franca (Brescia)		30		104			58			
	23.06.2013	Montebelluna - via Bonulovanni 92						1198			17	
	26.06.2013	Montebelluna						496				
	27.06.2013	Montebelluna		43				1316	11		31	16
	16.06.2013	Cisterna d'Asti, Valle San Matteo		26				533	302	24	454	
	23.06.2013	Cisterna d'Asti, Valle San Matteo		31				37	31		16	
	30.06.2013	Cisterna d'Asti, Valle San Matteo		37					18		12	
	07.07.2013	Cisterna d'Asti, Valle San Matteo		11				10	14		17	
Luxembourg	04.06.2013	Municipality of Biwer, E Lux										
	01.06.2013	Municipality of Manternach										
	03.06.2013	Municipality of Clemency										
	28.05.2013	Municipality of Clemency										
	04.06.2013	Municipality of Linger										

Table A5 (continued):

Country	Date sampled	Location				Fung	jicides (ug/kg po	llen)*			
			Fam.	F'hex.	F'pro.	F'diox.	F'sil.	Folpet	lprov.	K-met	M/M	Myclo.
Poland	28.05.2013	GA,Siorowo, Oziatdowo County Warminsko-Mazurskie					34					
	28.05.2013	Jankowice, Dziatdowo county, Warminsko										
	14.06.2013	Miedzna slaskie										
	12.06.2013	Sinki										
	16.06.2013	Stawiec										
	28.05.2013	Matujowice										
	30.05.2013	Matujowice Koto Brzegu										
Romania	16.06.2013	Daia Română (Alba County)										
	25.06.2013	Hârșești (Argeș County)										
	26.06.2013	Pașcani (lași County)										
	28.06.2013	Şuştra (Timiş County)										
	29.07.2013	Drogu-Udrești (Brăila County)										
	30.07.2013	Cilibia (Buzău County)										
	02.08.2013	Făcăieni (lalomița County)										
	07.08.2013	Gostinu (Giurgiu County)										
	07.08.2013	Badeana (Vaslui County)		13				51				
	20.08.2013	Moara Vlasiei (Ilfov County)										
Spain	28.07.2013	La Santa Espina (Castromonte) en Valladolid										
	28.07.2013	La Santa Espina (Castromonte) en Valladolid										
	20-21.08.2013	Hombrados (Guadalajara)										
	30.07.2013	Argamasilla de Alba (Ciudad Real)										
	30.07.2013	Argamasilla de Alba (Ciudad Real)										41
	30.07.2013	Llanos de Caudillo (Ciudad Real)										27
	29.07.2013	Cádiz										
	12.08.2013	Alcázar del Rey (Cuenca)										
	13.08.2013	Bocairent (Valencia)										
	05.08.2013	Cabezas de San Juan (Sevilla)										
	17.07.2013	Córdoba (no entiendo el municipio)										
	19.08.2013	Montoro (Sevilla)										
	08.08.2013	Tarazona de la Guareña (Salamanca)										
	25.08.2013	Plana de Alcañiz (Teruel)										
Sweden	05.07.2013	Vikbolandet										
	03.07.2013	Vikbolandet								28		

Table A5 (continued):

Country	Date sampled	Location				Fung	icides (µ	ug/kg po	llen)*			
			Fam.	F'hex.	F'pro.	F'diox.	F'sil.	Folpet	lprov.	K-met	M/M	Myclo.
Switzerland	06.05.2013	Laupersdorf										
	08.05.2013	Ogens										
	13.05.2013	Ecublens										
	07.05.2013	Brent					973					
	21.05.2013	Wabern bei Bern										
	23.05.2013	Melchnau										
	01.06.2013	Melchnau										
	18.08.2013	Laupersdorf										
	15.04.2013	Attiswil										
	02.09.2013	Attiswil										

Fam. Famoxadone F'hex. Fenhexamid F'pro. Fenpropimorph F'diox. Fludioxonil F'sil. Flusilazole Folpet Folpet Iprovalicarb lprov. K-met . Kresoxim-methyl M/M Metalaxyl / Metalaxyl-M Myclo. Myclobutanil

Table A5 (continued):

Country	Date sampled	Location			1	Fungicid	es (µg/k	g pollen)	*		
			Pen.	Pyrac.	Pyri.	Quin.	Spiro.	Tebuc	Thi-m.	Tolyl.	Triflox
Austria	09.05.2013	Styria, Fürstenfeld									
	09.05.2013	Styria, Fürstenfeld									
	May 2013	Grieskirchen, Rottenbach, upper Austria						30			
France	14.04.2013	Rhöne-Alpes SE France (near Lyon)							24		
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)						159			
	24.04.2013	Midi-Pyrénées SW France (near Toulouse)									
	19.06.2013	Ain-East Center France, north from the Alps									
	26.06.2013	Ain-East Center France, north from the Alps									
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"									
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse									
	31.07.2013	Midi-Pyrénais, SW France, near Toulouse									
	01.08.2013	Pyrenees, S France									
	28.08.2013	Midi-Pyrénais, SW France, near Toulouse									
	03.08.2013	SW France, bet.Bordeaux & Toulouse, "Lot-et-Garonne"									
	End of July	Ain-East Center France, north from the Alps									
Germany	22.05.2013	Rheinberg									
	11.06.2013	21521 Dassendorf		32							1104
	30/31.05.2013	21465 Wentorf									
	15.05.2013	Sachsen, OT Großbardan, botanical reserve Totenberg									
	28.05.2013	23847 Pölitz									
	20.05.2013	Baden-Württemberg, 88521 Ertingen									
	15/16.05.2013	Lower Saxony, 31241 Kleine Ilsede									
	22.05.2013	Baden Württemberg, 68809 Neulaßheim									
	29.05.2013	Baden-Württemberg, 71717 Beilstein					10				
	19.05.2013	Nordrhein Westfalen, 48324 Sendenhorst									
	21.05.2013	Saxony, 09123 Chemnitz									
	02.06.2013	Baden Württemberg, 72348 Rosenfeld									
	15.05.2013	Baden Württemberg, 74626 Bretzfeld									26
	15/16.05.2013	Saxony, 01683 Nossen							17		
	06.06.2013	Schleswig Holstein, 25899 Herrenkrog									

Table A6:

Country	Date sampled	Location	Fungicides (µg/kg pollen)*									
			Pen.	Pyrac.	Pyri.	Quin.			Thi-m.	Tolyl.	Triflox	
Greece	27.06.2013	Trikala / Thessalia										
	28.06.2013	Voiotia										
	28.06.2013	Voiotia										
	01.07.2013	Arta/ Epiros										
	28.06.2013	Halkidiki										
	28.06.2013	Halkidiki										
	16.07.2013	Soufli, Evros										
	28.06.2013	Redestos, Thessaloniki										
	28.06.2013	Redestos, Thessaloniki										
	19.07.2013	Lagadas, Thessaloniki										
Hungary	28.05.2013	Kisbodak										
	10.07.2013	Siófok										
	28.05.2013	Nagykónyi										
	10.07.2013	Nagykónyi										
	28.05.2013	Siófok										
	10.07.2013	Egyházaskozàr										
	10.07.2013	Tab										
Italy	15.05.2013	Ponte in Valtellina - Loc. S.Rocco			16							
	02.06.2013	Corte Franca (Brescia)					13				100	
	07.06.2013	Corte Franca (Brescia)				23	13				49	
	13.06.2013	Corte Franca (Brescia)				19	13				37	
	19.06.2013	Corte Franca (Brescia)										
	23.06.2013	Montebelluna - via Bonulovanni 92					22					
	26.06.2013	Montebelluna										
	27.06.2013	Montebelluna	13			25	13					
	16.06.2013	Cisterna d'Asti, Valle San Matteo	102				83	296	29		220	
	23.06.2013	Cisterna d'Asti, Valle San Matteo						29			22	
	30.06.2013	Cisterna d'Asti, Valle San Matteo						22			72	
	07.07.2013	Cisterna d'Asti, Valle San Matteo					12				47	
Luxembourg	04.06.2013	Municipality of Biwer, E Lux										
	01.06.2013	Municipality of Manternach										
	03.06.2013	Municipality of Clemency										
	28.05.2013	Municipality of Clemency										
	04.06.2013	Municipality of Linger										

Table A6 (continued):

Country	Date sampled	Location	Fungicides (µg/kg pollen)*										
			Pen.	Pyrac.	Pyri.	Quin.			Thi-m.	Tolyl.	Triflox		
Poland	28.05.2013	GA,Siorowo, Oziatdowo County Warminsko-Mazurskie											
	28.05.2013	Jankowice, Dziatdowo County, Warminsko							10				
	14.06.2013	Miedzna slaskie											
	12.06.2013	Sinki							68				
	16.06.2013	Stawiec											
	28.05.2013	Matujowice						16					
	30.05.2013	Matujowice Koto Brzegu											
Romania	16.06.2013	Daia Română (Alba County)											
	25.06.2013	Hârşeşti (Argeş County)											
	26.06.2013	Pașcani (lași County)							93				
	28.06.2013	Şuştra (Timiş County)							27				
	29.07.2013	Drogu-Udrești (Brăila County)											
	30.07.2013	Cilibia (Buzău County)											
	02.08.2013	Făcăieni (lalomița County)											
	07.08.2013	Gostinu (Giurgiu County)											
	07.08.2013	Badeana (Vaslui County)											
	20.08.2013	Moara Vlasiei (Ilfov County)											
Spain	28.07.2013	La Santa Espina (Castromonte) en Valladolid											
	28.07.2013	La Santa Espina (Castromonte) en Valladolid											
	20-21.08.2013	Hombrados (Guadalajara)											
	30.07.2013	Argamasilla de Alba (Ciudad Real)											
	30.07.2013	Argamasilla de Alba (Ciudad Real)											
	30.07.2013	Llanos de Caudillo (Ciudad Real)											
	29.07.2013	Cádiz											
	12.08.2013	Alcázar del Rey (Cuenca)											
	13.08.2013	Bocairent (Valencia)											
	05.08.2013	Cabezas de San Juan (Sevilla)											
	17.07.2013	Córdoba (no entiendo el municipio)											
	19.08.2013	Montoro (Sevilla)											
	08.08.2013	Tarazona de la Guareña (Salamanca)											
	25.08.2013	Plana de Alcañiz (Teruel)											
Sweden	05.07.2013	Vikbolandet											
	03.07.2013	Vikbolandet											

Table A6:

Country	Date sampled	Location	Fungicides (µg/kg pollen)*										
			Pen.	Pyrac.	Pyri.	Quin.	Spiro.	Tebuc	Thi-m.	Tolyl.	Triflox		
Switzerland	06.05.2013	Laupersdorf							21				
	08.05.2013	Ogens											
	13.05.2013	Ecublens											
	07.05.2013	Brent			169								
	21.05.2013	Wabern bei Bern											
	23.05.2013	Melchnau											
	01.06.2013	Melchnau											
	18.08.2013	Laupersdorf											
	15.04.2013	Attiswil											
	02.09.2013	Attiswil								44			

Penconazole Pen. Pyrac. Pyraclostrobin Pyri. Pyrimethanyl Quin. Quinoxyfen Spiro. Spiroxamine Tebuc Tebuconazole Thiphanate-methyl Thi-m Tolyl. . Tolylfluanid Triflox Trifloxystrobin

Table A6 (continued):

Country	Date sampled	Location	Insecticides/acaricides (µg/kg beebread)										
			lmid.	Cloth.	Thiam	Acrin.	Amit.	Bupro.	Ch(-e)	Cou.	tau-F	Piir.	Prop.
Austria	06.03.2013	Lower Austria /Zöfing (Danube flood plain)											
	05.03.2013	Lower Austria/ großer Grund - Donauauen close to Zwentendorf											
	03.09.2013	Grieskirchen, Rottenbach, Upper Austria									76		
	03.09.2013	Grieskirchen, Upper Austria											
	03.09.2013	Grieskirchen, Upper Austria											
France	11.03.2013	Rhöne-Alpes South East France (near Lyon)									93		
	22.03.2013	Vendée (85) West France, Niort-la Rochelle					503						
	27.03.2013	Midi-Pyrénées south west France (near Toulouse)											
Germany	22.03.2013	Northern Germany; Lower Saxony											
	22.03.2013	Northern Germany; Lower Saxony											
	10.06.2013	Baden Württemberg, 68809 Neulaßheim											
Hungary	13.04.2013	Kiskunhalas									98		
	13.04.2013	Kiskunhalas								148			
	13.04.2013	Kiskunhalas											
Poland	22.04.2013	Miedzna					137						
	27.03.2013	Kujawsko-Pomorskie							13				
	27.03.2013	Kujawsko-Pomorskie											
Spain	21.03.2013	Seville								12073	11		
	22.03.2013	Gilena, Andalucia						10	99	204	13	16	26
	22.04.2013	Guadalajara / Hombrados				22					11		
Switzerland	02.04.2013	Aedarmannsdorf											
	15.04.2013	Attiswil					177						
	17.04.2013	Heimenhausen					61						
	17.04.2013	Dietikon					31						
	17.04.2013	Dietikon					137						
						* Kev to							

Table A7:

Full data for concentrations of certain insecticides/acaricides in beebread samples collected during 2013, showing sampling dates and locations.

Country	Date sampled	Location	Fungicides (µg/kg beebread)									
			Bosc.	Carb.	Cypr.	Dim.	F'hex.	F'diox.	Folpet	Tebuc.		
Austria	06.03.2013	Lower Austria /Zöfing (Danube flood plain)										
	05.03.2013	Lower Austria/ großer Grund - Donauauen close to Zwentendorf										
	03.09.2013	Grieskirchen, Rottenbach, Upper Austria										
	03.09.2013	Grieskirchen, Upper Austria										
	03.09.2013	Grieskirchen, Upper Austria										
France	11.03.2013	Rhöne-Alpes South East France (near Lyon)										
	22.03.2013	Vendée (85) West France, Niort-la Rochelle				37			92			
	27.03.2013	Midi-Pyrénées south west France (near Toulouse)										
Germany	22.03.2013	Northern Germany; Lower Saxony			18			17				
	22.03.2013	Northern Germany; Lower Saxony										
	10.06.2013	Baden Württemberg, 68809 Neulaßheim					13					
Hungary	13.04.2013	Kiskunhalas										
	13.04.2013	Kiskunhalas		14						27		
	13.04.2013	Kiskunhalas										
Poland	22.04.2013	Miedzna	12		64			129				
	27.03.2013	Kujawsko-Pomorskie										
	27.03.2013	Kujawsko-Pomorskie										
Spain	21.03.2013	Seville										
	22.03.2013	Gilena, Andalucia		153								
	22.04.2013	Guadalajara / Hombrados										
Switzerland	02.04.2013	Aedarmannsdorf										
	15.04.2013	Attiswil										
	17.04.2013	Heimenhausen										
	17.04.2013	Dietikon										
	17.04.2013	Dietikon										

Boscalid Bosc. Carb. Carbendazim Cypr. Cyprodinil Dim. Dimethomorph F'hex. Fenhexamid . F'diox. Fludioxonil Folpet Folpet Tebuconazole Tebuc

Table A8:

Full data for concentrations of certain fungicides in beebread samples collected during 2013, showing sampling dates and locations.

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For more information contact:

Greenpeace Research Laboratories School of Biosciences Innovation Centre Phase 2 Rennes Drive University of Exeter Exeter EX4 4RN United Kingdom