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A Survey of Pesticide Residues in Pollen Loads Collected by Honey Bees in France

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ABSTRACT In 2002, a field survey was initiated on French apiaries to monitor weakness of honey bee, *Apis mellifera* L., colonies. Apiaries were evenly distributed in five sites located on continental France. Five colonies were randomly selected in each apiary, leading to a total of 125 studied honey bee colonies. For 3 yr (starting in autumn 2002), colonies were visited four times per year: after winter, before summer, during summer, and before winter. Pollen loads from traps were collected at each visit. Multiresidue analyses were performed in pollen to search residues of 36 different molecules. Specific analyses were conducted to search fipronil and metabolites and also imidacloprid and metabolites. Residues of 19 searched compounds were found in samples. Contamination by pesticides ranged from 50 to 0%. Coumaphos and tau-fluvalinate residues were the most concentrated of all residues (mean concentrations were 925.0 and 487.2 μg/kg, respectively). Fipronil and metabolite contents were superior to the limit of detection in 16 samples. Residues of fipronil were found in 10 samples. Nine samples contained the sulfone compound, and three samples contained the desulfinyl compound. Residues of imidacloprid and 6-chloronicotinic acid were found in 69% of samples. Imidacloprid contents were quantified in 11 samples with values ranging from 1.1 to 5.7 μg/kg. 6-Chloronicotinic acid content was superior to the limit of quantification in 28 samples with values ranging from 0.6 to 9.3 μg/kg. Statistical tests showed no difference between places of sampling with the exception of fipronil. Possible origins of these contaminations, concentration and toxicity of pesticides, and the possible consequences for bees are discussed.

KEY WORDS honey bees, pesticides, pollen loads, pollution

The use of honey bee *Apis mellifera* L. (Hymenoptera: Apidae), as a tool for monitoring environmental pollution has been discussed in previous studies. The insect effectiveness as an ecological detector is founded upon several ethological features such as high rate of reproduction, great mobility, large flying range, and numerous flower inspections per day. It is also founded on morphological characteristics: the honey bee body is covered with hairs that collect various particles and increase by this means the contact of the insect with its environment (Porrini et al. 2002).

Apicultural matrix analysis such as honey, wax, bees themselves, or pollen can provide useful indications of the diffusion of pesticides within the environment. Because honey is a product of human consumption, its contamination has been studied in many countries such as Portugal and Spain (Blasco et al. 2003), Germany (Wallner 1999), Belgium (De Greef et al. 1994), Greece (Thrasyvoulou and Pappas 1988, Balayannis and Santas 1992), and Switzerland (Bogdanov et al. 1999, Balayannis 2001). Every year in France, a national scheme evaluates commercial honey contamination by antibiotics (chloramphenicol, tetracycline, and sulfathiazole), acaricides (fluvalinate, bromopropylate, amitraze, and coumaphos), and heavy metals (lead and cadmium). In 2003, of 117 honey samples analyzed, 2.75% were not in keeping with the legal threshold (maximal residue limit) (Direction Générale de l'Alimentation 2004).

Wax and honey bees also have been subjected to various analyses to detect different types of contamination. Chemical contamination through *Varroa* treatments (*Varroa destructor* Anderson & Trueman [Acari: Mesostigmata]) has been found to be a route of wax contamination by coumaphos (Tremolada et al. 2004), bromopropylate (Hansen and Petersen 1988), and fluvalinate (Tsigouri et al. 2004). In addition, numerous studies have reported the use of honey bees to monitor environmental radionuclide contamination (Haarmann 1997, Barisic et al. 2002) or heavy metal contamination (Porrini et al. 2002). Less commonly, honey bees have been used as bioindicators to detect the presence of phytopathogenic microorganism in the environment (Porrini et al. 2002).

There are three main purposes for monitoring bee products: consumer health protection, international

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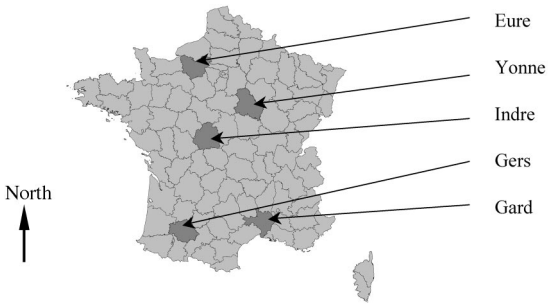

Fig. 1. Localization and names of the surveyed French sites.

commercial competition, and better product quality (Yakobson 1996). Although pollen loads are a product for human consumption, they currently are subject to no legislation, leading to very limited data. For experimental purposes, pollen loads collected from traps set on honey bee colonies have occasionally been used to monitor environmental pollution: radioactive contamination after the Chernobyl accident (Barisic et al. 1994) or new insecticide uses for particular crops, such as sweet corn (Erickson et al. 1997) or oilseed rape (Kevan et al. 1984, Fries and Wibran 1987). Pollen also has often been analyzed for the presence of pesticide residues in bee mortalities (Johansen and Brown 1972, Waller et al. 1984, Kubik et al. 1999). Samples were either collected with the aid of pollen traps or more often by hand, directly from flowers.

To assess the contamination resulting from pesticide uses in agriculture and their consequences on beehive health, a field survey was initiated in France in 2002. This investigation aimed to evaluate the impact of the bee weakness phenomenon in French apiaries. Only the results on pesticide residues in pollen loads are presented here. To our knowledge, no survey has been carried out during a whole year on the level of pesticides in pollen collected by honey bees.

Materials and Methods

The surveyed apiaries were distributed in five sites located in continental France (Fig. 1). In each site, five apiaries were chosen depending on their environment. In each apiary, five colonies randomly selected were visited four times a year (i.e., 125 colonies in total). Clinical data are not shown in this article. Two extra colonies fitted with pollen traps were chosen for pollen collection. Subsequent chemical searches were conducted on pollen samples collected from these colonies. Beekeepers were asked to set pollen traps in function 1 wk before each visit. Pollen loads were gathered in paper envelops by the beekeepers every 2 or 3 d, depending on the season and immediately frozen. After collection from the beekeepers' freezers, samples were transported in a coolbox and were stored in the laboratory at −20°C until analysis.

Visits took place at the following times of the year: before winter (October–November 2002), after winter (March–April 2003), before summer (May–June 2003), during summer (July–August 2003), and before winter (October 2003).

Professional and nonprofessional beekeepers were asked to follow their usual apicultural methods. However, for practical reasons, they were asked to leave the surveyed colonies in the same location year-round (no migratory beekeeping was allowed). Beekeepers were interviewed regarding their apicultural practices and any problems they encountered. Particular attention was paid to treatments against *V. destructor.* Any other treatment given to colonies also was reported.

Chemical Analyses. Analysis were performed in two laboratories: Agence Française de Sécurité Sanitaire des Aliments (AFSSA) laboratory, Sophia-Antipolis, France, and Groupement Interrégional sur les Recherches des Produits Agropharmaceutiques (GIRPA) laboratory, Angers, France. Residues of 36 contaminants were searched through multiresidue analysis. Pesticide family, purpose of use, and selling allowance in France in 2003 are detailed in Table 1. Residues of 41 different molecules were searched through individual or multiresidue analysis: 34 molecules were active ingredients of commercial preparations, and seven molecules were metabolites. These pesticides were chosen because of their high toxicity toward honey bees or because of their frequent uses in the field. Among the 34 active substances, 25 were insecticides and nine were fungicides. Other purposes for using these active substances were their acaricide (three), growth regulator (one), nematicide (one), or molluscicide (two) characteristics (Table 1). The use of 31 active substances from the list was legally authorized in 2003. Two pesticides were banned for plant protection uses (coumaphos and lindane).

Pollen samples from the two collecting hives were merged, resulting in one pollen sample per apiary. Two subsamples were made, one subsample for each laboratory. Appropriate quantities were then taken for analyses: 10 g for imidacloprid and 6-chloronicotinic acid, 1 g for fipronil and metabolites, 2 g for the multiresidue analysis conducted in the GIRPA laboratory, and 10 g for the analysis conducted in the AFSSA laboratory.

Imidacloprid and 6-cloronicotinic acid were searched through specific analyses. Samples were extracted with methanol and water added with diluted sulfuric acid. After filtration, an aliquot was concentrated down to the aqueous residue. This extract was subsequently diluted, washed with *n*-hexane, and cleaned up on an Amberlite XAD-4 cartridge. The resulting extract was divided into two equal portions, one portion for imidacloprid residues determination, and the other portion for total residues (i.e., 6-cloronicotic acid) determination. For imidacloprid residue analysis, the extract was concentrated, dissolved in water, and cleaned up with dichloromethane on a Chem-Elut column.

Quantification was conducted using liquid chromatography-tandem mass spectrometry (LC/MS/MS). The second extract obtained after Amberlite XAD-4 cleanup was used to hydrolyze imidacloprid and its metabolites in 6-chloronicotinic acid. For 6-chloroni-

Table 1. Characteristics of the surveyed pesticides

Pesticide	Pesticide family	Purpose of use	Status in 2003	LOD	LOQ
6-Chloronicotinic acid	Metabolite of imidacloprid	I	A	0.2	0.6
Aldicarb	Carbamate	I, N	A	5.0	10.0
Aldicarb sulfoxide	Metabolite of aldicarb	NR	NR	5.0	10.0
Aldicarb sulfone	Metabolite of aldicarb	NR	NR	5.0	10.0
Azinphos-methyl	Organophosphorus	I	A	57.0	196.7
Carbaryl	Carbamate	I, G S	A	5.0	10.0
Carbofuran	Carbamate	I	A	5.0	10.0
Chlorpyrifos ethyl	Organophosphorus	I	A	10.0	34.5
Coumaphos	Organophosphorus	I, A	B	37.0	142.6
Cyfluthrin	Pyrethroids	I	A	7.0	98.7
Cypermethrin	Pyrethroids	I	A	3.8	93.3
Cyproconazole	Triazole	F	A	5.0	10.0
Deltamethrin	Pyrethroids	I	A	0.1	29.9
Dimetoate	Organophosphorus	I	A	18.0	59.6
Endosulfan	Organochlorine	I	A	0.1	8.0
Epoxyconazole	Triazole	F	A	5.0	10.0
Fenitrothion	Organophosphorus	I	A	19.0	66.9
Fenthion	Organophosphorus	I	A	8.0	30.6
Fipronil	Phenylpyrazole	I	A	0.3	2.0–0.5
Fipronil sulfone compound	Metabolite of fipronil	I	A	0.3	2.0–0.5
Fipronil desulfinyl compound	Metabolite of fipronil	I	A	0.3	2.0–0.5
Flusilazole	Triazole	F	A	5.0	10.0
Hexaconazole	Triazole	F	A	10.0	20.0
Imidacloprid	Neonicotinoid	I	A	0.2	1.0
Lindane	Organochlorine	I	B	0.1	4.0
Malathion	Organophosphorus	I	A	9.0	31.5
Mercaptodimethur	Carbamate	I, M	A	5.0	10.0
Mercaptodiméthur sulfone	Metabolite of mercaptodimethur	NR	NR	5.0	10.0
Mercaptodimethur sulfoxide	Metabolite of mercaptodimethur	NR	NR	5.0	10.0
Methidathion	Organophosphorus	I	A	13.0	49.6
Methomyl	Carbamate	A, I, M	A	5.0	10.0
Mevinphos	Organophosphorus	I	A	3.8	27.7
Myclobutanil	Triazole	F	A	5.0	10.0
Oxamyl	Carbamate	N	A	5.0	10.0
Parathion ethyl	Organophosphorus	I	A	8.0	30.4
Parathion methyl	Organophosphorus	I	A	10.0	39.5
Penconazole	Triazole	F	A	5.0	10.0
Propiconazole	Triazole	F	F	5.0	10.0
Tau-fluvalinate	Pyrethroids	I, A	A	1.1	76.0
Tebuconazole	Triazole	F	A	10.0	20.0
Tetraconazole	Triazole	F	A	5.0	10.0

Chemical family, purpose of use (A, acaricide; F, fungicide, G S, growth substance; I, insecticide; N, nematicide; M, molluscicide, NR, not relevant): legal status for agricultural use in 2003 (A, authorized; B, banned); LOD and LOQ in micrograms per kilogram.

cotinic acid residue determinations, the extract was acidified. Residues were extracted with tertiobutyl methyl-ether. The ether phase was dried. Residues were dissolved with acidified methanol and water to be cleaned up on an HLB cartridge. Quantification was conducted using LC/MS/MS. Limit of detection (LOD) was 0.2 μg/kg, and limit of quantification (LOQ) was 1.0 μg/kg.

Fipronil and metabolites also were searched through specific analyses. Samples were extracted with acetone. After filtration, the extract was evaporated to dryness. Dried residue was dissolved in methanol. This extract was subsequently purified on an alumine cartridge and a subsequent immunologic cartridge. The resulting extract was dissolved with *n*-dodecane and evaporated. Methanol and water were used to dissolve residues. Quantification was conducted using LC/MS/MS. Fipronil and two of its metabolites, sulfone and desulfinyl compounds were quantified using LC/MS/MS. LOD was 0.3 μg/kg for fipronil and metabolites. During the study, LOQs decreased from 2.0 to 0.5 μg/kg according to analytical method improvements (Table 1).

Two multiresidue analysis were conducted, one analysis for carbamates and fungicides and the other analysis for organophosphorus, organochlorine, and pyrethroid insecticide search. Samples for carbamate and fungicide analysis were extracted with ethyl acetate. After filtration, the extract was divided in two equal volumes and evaporated. One of the dried residues was dissolved in methanol and water, and the other residue was dissolved in methanol and water added with sodium acetate. Quantification was conducted on liquid chromatograph/mass spectrometer.

Samples for the second multiresidue analysis were extracted with acetone and subsequently with dichloromethane after a liquid/liquid separation. A cleanup step with silica gel was performed. The two fractions were concentrated by evaporation. Residues obtained were dissolved in iso-octane for gas chromatographic analysis. Multiresidue analysis was performed by gas chromatography by using an electron capture detec-

Table 2. Pesticide residues in pollen loads

Pesticide	No. analyzed samples	No. positive samples	%	Residue concn		Avg concn (μg/kg)
				min. (μg/kg)	max (μg/kg)	
Imidacloprid	81	40	49.4	>LOD	5.7	1.2
6-Chloronicotinic acid	81	36	44.4	>LOD	9.3	1.2
Fipronil	81	10	12.4	>LOD	<LOQ	1.2
Fipronil desulfynil compound	81	9	11.1	>LOD	1.5	1.3
Penconazole	79	8	10.1	>LOD	126.0	27.6
Carbaryl	36	3	8.3	126.0	265.0	218.7
Endosulfan	82	5	6.1	>LOD	340.0	81.2
Tau-fluvalinate	82	5	6.1	>LOD	2020.0	487.2
Flusilazole	79	4	5.1	>LOD	71.0	26.1
Parathion-methyl	82	4	4.9	>LOD	<LOQ	24.8
Carbofuran	79	3	3.8	>LOD	10.9	14.0
Cyproconazole	79	3	3.8	>LOD	<LOQ	7.5
Fipronil sulfone compound	81	3	3.7	1.7	3.6	1.2
Myclobutanil	72	2	2.8	>LOD	20.3	13.9
Coumaphos	82	2	2.4	150.0	1700.0	925.0
Oxamyl	55	1	1.8	38.4	38.4	38.4
Tebuconazole	79	1	1.3	12.3	12.3	12.3
Hexaconazole	79	1	1.3	18.0	18.0	18.0
Parathion-ethyl	82	1	1.2	>LOD	<LOQ	19.2
Aldicarb	79	0	0.0	ND	ND	ND
Aldicarb sulfoxide	24	0	0.0	ND	ND	ND
Aldicarb sulfone	40	0	0.0	ND	ND	ND
Azinphos-methyl	82	0	0.0	ND	ND	ND
Chlorpyrifos-ethyl	82	0	0.0	ND	ND	ND
Cyfluthrin	82	0	0.0	ND	ND	ND
Cypermethrin	82	0	0.0	ND	ND	ND
Deltamethrin	82	0	0.0	ND	ND	ND
Dimetoate	82	0	0.0	ND	ND	ND
Epoxyconazole	79	0	0.0	ND	ND	ND
Fenitrothion	82	0	0.0	ND	ND	ND
Fenthion	82	0	0.0	ND	ND	ND
Lindane	82	0	0.0	ND	ND	ND
Malathion	82	0	0.0	ND	ND	ND
Mercaptodimethur	73	0	0.0	ND	ND	ND
Mercaptodiméthur sulfone	71	0	0.0	ND	ND	ND
Mercaptodimethur sulfoxide	73	0	0.0	ND	ND	ND
Methidathion	82	0	0.0	ND	ND	ND
Methomyl	43	0	0.0	ND	ND	ND
Mevinphos	82	0	0.0	ND	ND	ND
Propiconazole	79	0	0.0	ND	ND	ND
Tetraconazole	79	0	0.0	ND	ND	ND

Pesticides are classified by decreasing frequencies (percentages). ND, not detected.

tor for organochlorine and pyrethroid pesticides and a nitrogen-phosphorus detector for organophosphorus pesticides. LODs for the different molecules searched through multiresidue analysis ranged from 0.1 to 57.0 μg/kg. LOQs ranged from 4.0 to 196.7 μg/kg (Table 1).

Statistical Analysis. For analysis of pesticide presence, we compared either frequencies (proportions of positive samples) or the average contents. In Table 2, percentages of polluted pollen samples were calculated by dividing the number of positive samples (samples where the selected compound was detected) by the total number of samples analyzed for this compound. This total number varied with the type of analysis and depended on the season because quantities of collected pollen were not always sufficient to perform all analyses. Pesticides were classified in decreasing order of frequencies in pollen samples (Table 2).

The average content of a sample was evaluated by the mean, using the arithmetic median when content values were between LODs and LOQs, rather than logarithmic median. For example, provided that tau-fluvalinate LOD was 1.1 μg/kg and LOQ equalled 76.0 μg/kg, the median was 38.6 μg/kg.

Statistical tests were conducted on frequencies, not on the pesticide contents. Logistic regression was used to describe the relationship between the dummy variable (presence or absence) and explicative variables (date or place of sampling). This model allows to estimate the probability that an event could occur when the explicative variable is known: P(Y X1, X2, . . . Xp). Likelihood ratio and the type III tests were used to estimate the coefficient of the model. When those two indicators pointed out that the effect (date or place of sampling) was not significant, subsequent tests were not pursued. When conditions of application of logistic regression were not fulfilled (separated data), Fisher exact probability and chi-square tests were performed. Unless otherwise stated, the significance threshold was 5%. All tests were performed using SAS System for Windows, version 8.

Results

Distribution and Concentration of Surveyed Pesticides in Pollen Loads. Among the 41 searched compounds, 19 were found in pollen loads. The most frequent residues were imidacloprid (49.4% of samples) 6-chloronicotinic acid (44.4%), and fipronil (12.4%). The proportion of samples with either imidacloprid, 6-chloronicotinic acid, or both was 69.1%. Maximum concentrations found in these positive samples were 5.7 and 9.3 μg/kg for imidacloprid and 6-chloronicotinic acid, respectively. Frequency of other pesticides residues (16 different compounds) ranged from 11.1 to 1.2% (Table 2).

The maximum concentration of two pesticides (coumaphos and tau-fluvalinate) were at the level of milligrams per kilogram (i.e., 1,000-fold the unit used to express LOQs, micrograms per kilogram). It is worth noting that these pesticides are acaricides used for *Varroa* treatment (Table 2).

Among the 101 pollen samples analyzed in this study, 73 samples were analyzed for the search of all the 41 compounds. Only nine samples (12.3%) were found containing no pesticide residues. Pollen loads were polluted with one to five different compounds. The highest frequency of contamination (31.5%) corresponded to the presence of one molecule. Samples polluted with two, three, or four active ingredients had the respective frequencies of 28.8, 20.6, and 5.5%. Only one sample was polluted with five compounds.

Time Distribution of Pollen Load Contamination. Frequencies of pollen contamination were plotted according to time of collection (Fig. 2). Eighty-one samples of pollen loads were analyzed for fipronil and its metabolite (sulfone compound and desulfinyl compound). Residue contents were superior to the LOD for one of the three searched compounds at least in 16 samples. Residues of the active ingredient fipronil were found in 10 samples. Nine samples contained the sulfone compound, and three samples contained the desulfinyl compound.

A peak of fipronil presence (i.e., pollen loads polluted by either fipronil or fipronil metabolites, or both) was observed in samples collected during March and April 2003 (45.8%), which was significantly different from rates of contamination from samples collected in November 2002 (22.2%), May–June (4.2%), July–August 2003 (5.9%), and September–October 2003 (14.3%) (type 3 analysis of effects: calculated $\chi^2 = 12.1$, df = 4, $\alpha = 0.02$; analysis of maximum likelihood estimates: calculated likelihood $\chi^2 = 10.4$, df = 1, $\alpha = 0.001$). No significant differences between frequencies were found regarding the place of sampling (calculated $\chi^2 = 1.5$, df = 4, $\alpha = 0.82$).

Residue contents were superior to LOQs in four samples (Table 3). The active ingredient fipronil has never been quantified, whereas the sulfone compound has been quantified three times and the desulfinyl compound a single time (1.5 μg/kg, sample collected during the summer visit). Sulfone compound contents ranged from 1.7 to 3.7 μg/kg. These samples were collected during the March–April, May–June, and September–October 2003 visits.

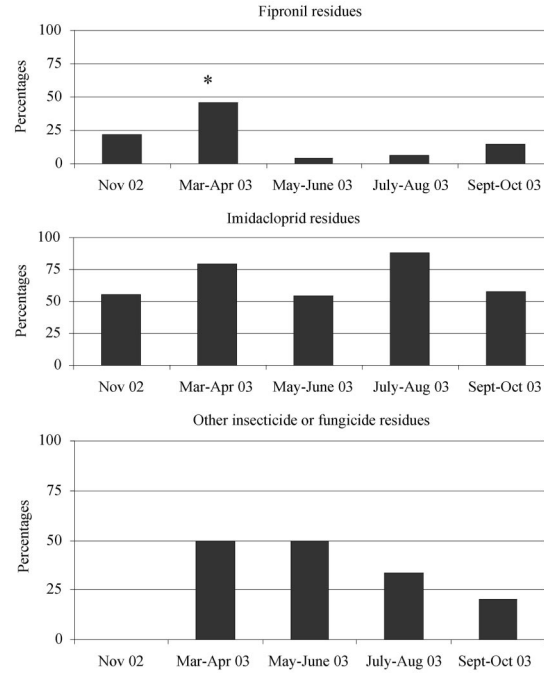

Fig. 2. Frequency (%) of fipronil (top), imidacloprid (middle), and other pesticides in pollen loads (bottom). Samples were considered positive when residues were superior to the LOD. LOD for fipronil and metabolites was 0.3 μg/kg. LOD for imidacloprid and metabolites was 0.2 μg/kg.

Search of imidacloprid and 6-chloronicotinic acid residues was conducted on the same 81 samples of pollen loads. In 56 samples, residues of one molecule at least have been found. Residues of imidacloprid were found in 40 samples, and residues of 6-chloronicotinic acid in 36 samples. Samples polluted with imidacloprid, 6-chloronicotinic acid, or both molecules were equally collected during the five sampling periods. Frequencies of contamination were 55.6, 79.2, 54.2, 88.2, and 57.1% for pollen loads sampled in No-

Table 3. Number of pollen load samples where concentrations of fipronil or its metabolite residues were superior to the LOQ

Sampling time	No. analyzed samples	Fipronil	Sulfone compound	Desulfinyl compound
Nov. 2002	9	0	0	0
Mar.–April 2003	24	0	1 (3.3)[a]	0
May–June 2003	24	0	1 (3.7)[a]	0
July–Aug. 2003	17	0	0	1 (1.5)[b]
Sept.–Oct. 2003	7	0	1 (1.7)[c]	0
Total	81	0	3	1

Residue contents are given in parentheses in micrograms per kilogram.
[a] LOD = 0.3 μg/kg; LOQ = 2.0 μg/kg.
[b] LOD = 0.3 μg/kg; LOQ = 1.0 μg/kg.
[c] LOD = 0.3 μg/kg; LOQ = 0.5 μg/kg.

Table 4. Number of pollen load samples where imidacloprid or its metabolites residue concentrations were superior to the LOQ

Sampling time	No. analyzed samples	Imidacloprid (min.; max)	6-Chloronicotinic acid (min.; max)
Nov. 2002	9	0	1 (2.8)
Mar.–April 2003	24	4 (2.7; 4.6)	6 (0.6; 3.0)
May–June 2003	24	2 (1.2; 1.2)	4 (0.7; 1.2)
July–Aug. 2003	17	4 (1.1; 5.7)	15 (0.8; 9.3)
Sept.–Oct. 2003	7	1 (1.4)	2 (1.1; 2.8)
Total	81	11	28

Residue contents are given in parentheses in micrograms per kilogram.

vember 2002, March and April 2003, spring 2003, summer 2003, and autumn 2003, respectively. Statistical tests have shown no significant differences in the contamination frequency (calculated $\chi^2 = 7.1$, df = 4, $\alpha = 0.12$). Tests also showed no statistical difference between sampling places.

Imidacloprid contents were quantified in 11 samples (Table 4). Values ranged from 1.1 to 5.7 μg/kg. 6-Chloronicotinic acid contents were superior to the LOQ in 28 samples, values ranging from 0.6 to 9.3 μg/kg.

Rates of contamination from other pesticides ranged from 50% (samples collected in March and April 2003 and in May and June 2003) to 0% (samples collected in November 2002). Statistical tests showed no difference between these sampling dates (calculated $\chi^2 = 8.4$, df = 4, $\alpha = 0.08$) and between places of sampling.

Discussion

The use of chemicals for insect pest management has come a long way since the indiscriminate use of organochlorine insecticides in the 1940s and 1950s. In France, ≈74,500 tons of pesticides (active substances) was used in 2003. Provided the cultivated surface of this country, France was found to be the first pesticide consumer in Europe in terms of tonnage but an average user in terms of quantities per surface in comparison with countries that consumed much more pesticides or far less as The Netherlands and Portugal, respectively (Devillers et al. 2005).

Routes of Pollen Load Contamination. Two acaricides used for *Varroa* control (coumaphos and tau-fluvalinate) were identified in pollen loads. Because coumaphos was banned for plant treatments in France in 2003 but authorized for animal and building treatments, its presence in pollen loads could reveal an illegal use. Experts on plant treatments stated that coumaphos was unlikely used in illegal plant management because its solubility in water is low. Because the two pollen samples contaminated with coumaphos residues originated from apiaries where beekeepers have stated using this active ingredient for *Varroa* control, one could reasonably assume that this pesticide has been introduced in pollen loads via honey bees. It is well known that honey bees use nectar and/or honey to glue pollen grains together while making pollen loads. The quantity of sugars added by bees in pollen loads can represent up to 40% of pollen load dry weight (Roulston and Cane 2000). However, this scenario cannot explain the presence of tau-fluvalinate in pollen loads because no beekeepers have reported the use of this active ingredient for *Varroa* control. The first reports of *Varroa* resistance to this molecule were published in 1995 (Faucon et al. 1995). Consequently, it has been advised not to use tau-fluvalinate in France since 2001 (Faucon et al. 2000). However, this advice is not always followed by beekeepers. Tau-fluvalinate also was widely used for plant protection in 2003 (Table 1). Therefore, its presence in pollen loads can be mainly or exclusively explained by agricultural use. This route of contamination could clarify the presence of every pesticides in pollen loads. In a study conducted in Italy between March and September 1995, pollen specimens were gathered weekly from two stations situated in an urban area and in a rural area. Although chemical treatments were forbidden during the flowering season, traces of one or more pesticides were found in more than one-third of the samples, even in periods that were not normal for field crop growing. From palynological analysis, it could be inferred that crops also had been treated in the presence of wind, causing pesticides to drift into surrounding areas (Porrini et al. 2002). Finally, another possible route of pollen load contamination could be the addition of polluted honey and nectar to paste pollen grains together, as demonstrated for coumaphos.

Concentration of Pesticides. In the current study, pesticide residues in pollen loads were found at concentrations much lower than the concentrations reported in the literature (Table 5). Parathion-methyl content had an average value of 24.8 μg/kg, which is lower than that found by Faucon et al. (1986) in France and those found by Waller et al. (1984) on sunflower pollen loads in the United States. However, Waller et al. (1984) observed no abnormal adult mortalities, no queen problems, nor atypical brood development at these high doses. Parathion-methyl residues also have been studied when the new microencapsulate technique was first used in the field, leading to intoxication of nontarget insects. Still in the United States, Russel et al. (1998) studied residues of >100 pesticides in apicultural matrixes. Although they did not list the searched pesticides, they reported that only parathion-methyl residues coming from microencapsulated preparations were found in pollen (Table 5).

In the current study, tau-fluvalinate residues were found with an average value of 487.2 μg/kg. In 1990, in a field experiment conducted in France by Haouar et al. (1990) on an apple tree orchards treated with fluvalinate, residues were found in pollen loads collected from traps. From the sixth day, no pesticide residue was found in pollen (Table 5). Various outdoors experiments such as open field and pollen collected within hives (Dahl and Lowell 1984) or semi field experimental set up to assess pesticide formula-

Table 5. Various pesticide contents in pollen found in the present study and in previous studies

Insecticide	Avg contents found in this study	Contents found in literature	Analytical methods	Reference
Azinphos-methyl	ND	260–590	LOD and LOQ NR	Dahl and Lowell 1984
Carbaryl	218.7	600	LOD and LOQ NR	Johansen and Brown 1972
		390–1,200	LOD and LOQ NR	Dahl and Lowell 1984
		7,100–94,000	LOD and LOQ NR	Kevan et al. 1984
Cyhalothrin	ND	10–500	LOD and LOQ NR	Fries and Wibran 1987
Cypermethrin	ND	70–1,900	LOD and LOQ NR	Fries and Wibran 1987
Parathion methyl	24.8	1,700–17,800	LOD and LOQ NR	Faucon et al. 1986
		40–1,940	LOD and LOQ NR	Waller et al. 1984
		10–2,470	LOD and LOQ NR	Russell et al. 1998
Tau-fluvalinate	487.2	5–260	LOD = 0.01 mg/g LOQ NR	Haouar et al. 1990

Contents are expressed in micrograms per larva. ND, not detected; NR, not reported.

tion toxicity (Kevan et al. 1984) have revealed high carbaryl residues in pollen. Johansen and Brown (1972) detected carbaryl residues in maize, *Zea mays* L., pollen collected within hive with a content of 600 μg/kg. This content apparently led to high colony mortalities in the state of Washington (Johansen and Brown 1972).

Several other pesticides searched in the current study were not found in pollen loads samples. However, literature mentions the possibility of finding traces of several other pesticides in the environment: azinphos-methyl in pollen collected within hives in the United States (Dahl and Lowell 1984) and cypermethrin and lambda-cyhalothrin in an experimental setup in Sweden (Fries and Wibran 1987). This last experiment also showed that residue levels were substantial the day of treatment, but they decreased rapidly the next few days. The absence of cypermethrin residues in pollen loads in our results could be explained by the low environmental persistence of pyrethroids. In conclusion, in the current study parathion methyl, carbaryl, azinphos methyl, and cypermethrin concentrations were lower than those in previous studies, whereas tau fluvalinate residues were higher.

Innovative methods of pesticide application and new commercial preparations are responsible for these obvious decreases in contents of pesticide residues in pollen loads. Historically, the efficiency of insecticide use, measured as the percentage of total chemicals applied to a crop that actually kills insects, has been shown to be woefully poor. Modern pest management had to address the problem of maximizing insecticide efficiency while minimizing waste. This ideal may be achieved by integrating several tactics, namely, choice of compound and concentration, and application timing and technology (Speight et al. 1999).

During the late 1970s and early 1980s, new techniques were developed in pest management. Among them, the production of microencapsulated insecticides was hailed as a giant step forward in reducing colony losses because of pesticide application (Robinson 1979). Then, systemic pesticides used as seed treatments were thought to be a solution to pulverization drift provided the active substance or metabolites would not be in contact with bees through nectar or pollen. As insecticides are developed with progressively higher potency, they are used in smaller amounts, which are more easily detoxified (Casida and Quistad 2004). In this study, fipronil and two of its metabolite residues were searched through specific analysis. In France, this systemic insecticide was thought to be responsible of bee colony losses by many apiarists and scientific teams. This active ingredient has been largely used for seed treatment because it provided long-term crop protection. This might be due, in part, to the combined action of the parent compound and the sulfone derivative, which was similar in potency to that of fipronil. Another factor might be the desulfinylated photoproduct whose toxicity toward house flies and mice has been found to be very close from that of fipronil (Hainzl and Casida 1996). When the three residues' frequencies were compared, our results suggested that the parent compound fipronil mostly occurred in pollen loads. The photoproduct had two possible origins. The first origin was the natural degradation when commercial preparations containing fipronil were used as aerial treatments. The second origin could be artificial exposition to light through the process of sampling. Because this survey did not focus on a specific culture, it is not possible to identify the origin of this metabolite.

In France in 2003, dispersal of fipronil in the aerial compartment was shown during sowing of sunflower seeds coated with fipronil preparation. The peak of pollen load contamination by fipronil and metabolites during March and April could be explained by this phenomenon.

Acute and Chronic Bee Exposure to Pesticides. No high honey bee mortalities (i.e., acute intoxication) were recorded during this study. However, the presence of various pesticide residues in pollen loads indicated that bee colonies were chronically exposed to xenobiotics (of the 73 samples analyzed for pesticides, only nine were found without any residues). Pollen loads are stocked by bees within the colony in the form of beebread, which is a mixture of honey, pollen, and several enzymes. Little is known about the future of pesticide residues in these conditions of storage, i.e., whether they are conserved as such or metabolized. Also, nothing is known on possible interactions be-

Table 6. Acute toxicity of pesticide for brood and for adult bees

Insecticide	Brood LD_{50}	Adult LD_{50}	Mode of administration	Reference
Aldicarb	0.356	0.272	U	Atkins and Kellum 1986
Aldicarbe sulfoxide	0.854	2.211	U	Atkins and Kellum 1986
Aldicarbe sulfone	1.067	399.3	U	Atkins and Kellum 1986
Azinphos-methyl	NA	NT	NR	Ghini et al. 2004
		0.428	U	University of California 1981
Carbaryl	1.212	1.34	U	Atkins and Kellum 1986
Carbofuran	NA	0.16	U	Ghini et al. 2004
Chlorpyrifos ethyl	NA	0.11	U	Ghini et al. 2004
Coumaphos	NA	3–6	O	Van Buren et al. 1992
Cypermethrin	0.066	0.060	U	Atkins and Kellum 1986
Deltamethrin	NA	0.7	O	Decourtye et al. 2004
Dimehoate	NA	0.18–0.90	U	Ghini et al. 2004
Endosulfan	28.142	21.79	U	Atkins and Kellum 1986
Fenitrothion	NA	0.28	U	Ghini et al. 2004
Fenthion	NA	0.30	U	Ghini et al. 2004
Fipronil	NA	0.004	0	Roper 2002
		0.006	T	
Imidacloprid	NA	0.0179	T	Iwasa et al. 2004
		0.04	O	Decourtye et al. 2003
Malathion	0.736	0.726	U	Atkins and Kellum 1986
Methidathion	0.274	0.237	U	Atkins and Kellum 1986
Methomyl	0.539	1.29	U	Atkins and Kellum 1986
Mevinphos	0.441	0.305	U	Atkins and Kellum 1986
Oxamyl	0.367	10.26	U	Atkins and Kellum 1986
Parathion ethyl	NA	0.07–0.10	T	Murray 1985
		0.09–0.13	O	
Parathion methyl	NA	0.29	U	Ghini et al. 2004
Tau-fluvalinate	NA	65.85	U	University of California 1981
Tebuconazole	NA	97–175.8	T	Schmuck et al. 2003

Brood LD_{50} are expressed in micrograms per larva, adult LD_{50} are expressed in micrograms per bee (NA, not available in literature; NT, nontoxic). For adults, pesticide mode of administration is mentioned as T for topical, O for oral, and U for unknown (NR, not relevant).

tween molecules present in the same sample of pollen or beebread. For example, in this study, residues of fungicides and pyrethroids in the same samples were found on one occasion. It is well known that some of these associations increase the toxicity of pesticides toward bees (Pilling and Jepson 1993).

Brood and adult bees are directly or indirectly (through the process of royal jelly secretion by hypopharyngeal glands) fed with beebread and can be exposed to pesticide residues for various times. Many research teams have worked on quantifying the amount of pollen needed to rear a bee larva. It mainly depends on season, pollen species, and conditions under which the larvae are reared. For example, it has been shown that worker honey bee larvae needed 86 mg of maize pollen for their complete development (Babendreier et al. 2004).

Pollen supply also is involved in bee resistance to pesticide exposure. Amount and quality of pollen ingested in the first days of life affected the pesticide sensitivity of young and older bees (Wahl and Ulm 1983). The most frequent residues (i.e., imidaclopride, 6-chloronicotinic acid, and fipronil) were searched with very low LODs compared with other pesticides. This finding is due to the specific analysis versus the multiresidue analysis. It is worth noting that pesticides found the most frequently in pollen samples were not the pesticides that had the highest residue concentrations (Table 2). For example, nearly one-half of pollen samples (49.4%) contained imidacloprid residues with an average concentration of 1.2 μg/kg. In contrast, only two samples contained coumaphos residues at an average concentration of 925 μg/kg. This discrepancy has to be put in perspective with the acute toxicity of active substances. Acute toxicity is measured through oral or contact lethal dose 50 (LD_{50}). Some LD_{50} values found in literature are reported in Table 6. Values are extremely variable according to the active ingredient. It also has been shown that LD_{50} values could vary with bee age, race, or temperature.

The main question to be addressed remains, Are the doses found in pollen dangerous for bees? Let us consider this question for imidacloprid. We would like to know how much contaminated pollen an adult bee should eat to reach the LD_{50} quantity. If we rely on the average content, we have found in this study (1.2 μg/kg) that the consumption of 33 g of pollen by one individual would be needed to meet the oral LD_{50} (0.04 μg per bee). A certain amount of time would be needed for one worker to eat this quantity of pollen (as mentioned above), which then would be stocked in cells in the form of bee bread. Our lack of knowledge on active ingredient fate and interactions between molecules stocked into hives was discussed above.

Moreover, it has been shown that LD_{50} values would not be sufficient to assess the adverse effects of a pesticide. Very small quantities of active ingredients can lead to subtle effects at various levels of bee physiology and behavior. These effects are more difficult to detect, but they also may affect bee populations. In chronic toxicity studies, imidacloprid reacts at

doses 60–6,000 times lower than those required to produce the same effect in acute intoxication studies (Suchail et al. 2001). For fipronil, an effect on the learning performances of bees has been shown in the range of 2.2–4.5 μg/liter in syrup that corresponded to the ingestion of 0.075 and 0.15 ng of active substance per bee per day, which represents 1/80th and 1/40th of the LD_{50}, respectively (Decourtye et al. 2005). If effects on several adults bees are added, one could except that the whole colony would be affected by the ingestion of very low doses of pesticide.

Brood LD_{50} also have been reported in Table 6. It is worth noting that values are different from adult LD_{50} for the same molecule. Under these conditions, data on toxicity assessment must be interpreted with care. Hazard of pesticides to bees is the result of exposition and toxicity. Exposition length of time (i.e., acute or chronic exposure) and stage of development (i.e., adult, larvae, or both stages) also play a large part in the effect of pesticides on a bee colony. More data are needed to state on the dangerousness of pesticide residues toward bee colonies on its own: data on pesticides presence within colonies (in bee bread and larvae) and also data on chronic exposure to single or association of pesticides.

In conclusion, this study has demonstrated the presence of a wide range of pesticides in pollen loads collected by honey bees. These pesticides were found at various concentrations from trace amounts to hundreds of micrograms per kilogram. This contamination was common year-round; no season was particularly more represented than another, with the exception of fipronil. All active molecules are ecotoxic, but one can expect large disparities in their potentials depending on their targets and their mode of action. Honey bees are exposed to these active substances by contact living in the colonies and also by feeding on them. Currently, it is rather difficult to comment on the impact of pesticides stocked within the hive by the mean of pollen loads. Little is known on pesticide fate in beebread. Although some studies had been aimed at assessing the effects of very small doses of pesticides on honey bee workers, how these very small doses would affect a whole colony deserves more study. One of the most enduring apicultural research problems will be the development of new techniques to evaluate in the field how pesticide contamination can affect honey bee individuals (workers, males, and queens) and colonies. The best way to evaluate such exposition would be to work on whole colonies, but this experimental solution is not yet available.

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BOOK REVIEW

Correction. In the article, "A Survey of Pesticide Residues in Pollen Loads Collected by Honey Bees in France," by Marie-Pierre Chauzat, Jean-Paul Faucon, Anne-Claire Martel, Julie Lachaize, Nicolas Cougoule, and Michel Aubert (99: 253–262), has a few errors in Table 2 on p. 256 (rows 4 and 13). See corrected Table 2 below:

Table 2. Pesticide residues in pollen loads

Pesticide	No. analyzed samples	No. positive samples	%	Residue concn		Avg concn (μg/kg)
				min. (μg/kg)	max (μg/kg)	
Imidacloprid	81	40	49.4	>LOD	5.7	1.2
6-Chloronicotinic acid	81	36	44.4	>LOD	9.3	1.2
Fipronil	81	10	12.4	>LOD	<LOQ	1.2
Fipronil sulfone compound	81	9	11.1	>LOD	3.7	1.6
Penconazole	79	8	10.1	>LOD	126.0	27.6
Carbaryl	36	3	8.3	126.0	265.0	218.7
Endosulfan	82	5	6.1	>LOD	340.0	81.2
Tau-fluvalinate	82	5	6.1	>LOD	2020.0	487.2
Flusilazole	79	4	5.1	>LOD	71.0	26.1
Parathion-methyl	82	4	4.9	>LOD	<LOQ	24.8
Carbofuran	79	3	3.8	>LOD	10.9	14.0
Cyproconazole	79	3	3.8	>LOD	<LOQ	7.5
Fipronil desulfinyl compound	81	3	3.7	>LOD	1.5	1.3
Myclobutanil	72	2	2.8	>LOD	20.3	13.9
Coumaphos	82	2	2.4	150.0	1700.0	925.0
Oxamyl	55	1	1.8	38.4	38.4	38.4
Tebuconazole	79	1	1.3	12.3	12.3	12.3
Hexaconazole	79	1	1.3	18.0	18.0	18.0
Parathion-ethyl	82	1	1.2	>LOD	<LOQ	19.2
Aldicarb	79	0	0.0	ND	ND	ND
Aldicarb sulfoxide	24	0	0.0	ND	ND	ND
Aldicarb sulfone	40	0	0.0	ND	ND	ND
Azinphos-methyl	82	0	0.0	ND	ND	ND
Chlorpyrifos-ethyl	82	0	0.0	ND	ND	ND
Cyfluthrin	82	0	0.0	ND	ND	ND
Cypermethrin	82	0	0.0	ND	ND	ND
Deltamethrin	82	0	0.0	ND	ND	ND
Dimetoate	82	0	0.0	ND	ND	ND
Epoxyconazole	79	0	0.0	ND	ND	ND
Fenitrothion	82	0	0.0	ND	ND	ND
Fenthion	82	0	0.0	ND	ND	ND
Lindane	82	0	0.0	ND	ND	ND
Malathion	82	0	0.0	ND	ND	ND
Mercaptodimethur	73	0	0.0	ND	ND	ND
Mercaptodiméthur sulfone	71	0	0.0	ND	ND	ND
Mercaptodimethur sulfoxide	73	0	0.0	ND	ND	ND
Methidathion	82	0	0.0	ND	ND	ND
Methomyl	43	0	0.0	ND	ND	ND
Mevinphos	82	0	0.0	ND	ND	ND
Propiconazole	79	0	0.0	ND	ND	ND
Tetraconazole	79	0	0.0	ND	ND	ND

Pesticides are classified by decreasing frequencies (percentages). ND, not detected.

Correction. In the article, "A Survey of Syrphid Predators of *Nasonovia ribisnigri* in Organic Lettuce on the Central Coast of California," by Hugh A. Smith and William E. Chaney (100: 39–48), authors would like to apologize for neglecting to acknowledge Robert Bugg of UC SAREP in the *Acknowledgments*.

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