Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera* L.) in France

Marie-Pierre Chauzat* and Jean-Paul Faucon

AFSSA, Les Templiers, 105 route des Chappes, BP 111- F-06 902 Sophia-Antipolis Cedex, France

Abstract: In 2002 a field survey was initiated in French apiaries in order to monitor the health of honey bee colonies (*Apis mellifera* L.). Studied apiaries were evenly distributed across five sites located in continental France. Beeswax samples were collected once a year over 2 years from a total of 125 honey bee colonies. Multiresidue analyses were performed on these samples in order to identify residues of 16 insecticides and acaricides and two fungicides. Residues of 14 of the searched-for compounds were found in samples. Tau-fluvalinate, coumaphos and endosulfan residues were the most frequently occurring residues (61.9, 52.2 and 23.4% of samples respectively). Coumaphos was found in the highest average quantities (792.6 $\mu g\,kg^{-1}$). Residues of cypermethrin, lindane and deltamethrin were found in 21.9, 4.3 and 2.4% of samples respectively. Statistical tests showed no difference between years of sampling, with the exception of the frequency of pyrethroid residues. Beeswax contamination was the result of both in-hive acaricide treatments and, to a much lesser extent, environmental pollution.

Keywords: beeswax; pesticide residues; environment; varroa treatment; France

1 INTRODUCTION

Contamination of bee products with pesticides has been widely documented for many years.[1,2] Pollution can be divided into environmental and apicultural sources, although some products can occur from both origins as they are used in both activities. Since the introduction of *Varroa destructor* (Anderson & Trueman) (Acari: Mesostigmata) into European colonies of honey bee (*Apis mellifera* L.), beekeepers have had to control the number of mites to prevent colony losses. Most of the time, acaricide treatments lead to residues in hives. Several surveys have already monitored residues in beeswax of acaricides such as bromopropylate,[3,4] coumaphos,[4] amitraz,[5] fluvalinate[4,5] and tetradifon, a compound mostly used in Asiatic countries.[4] The accumulation of pesticides in beeswax may also result from environmental pollution. However, very few references concerning beeswax contamination related to crop treatments are available in the literature.[2]

Results of surveys have shown how widely residues are present in beeswax and how they could potentially impact upon colony biology.[4] From an economic point of view, bee products should maintain the image of being natural, healthy and clean substances. Finally, the use of honey bees or honey bee products as a tool for monitoring environmental pollution has been discussed many times in previous studies.[6,7] Therefore, studying residues in beeswax is relevant to several apicultural issues: colony health, economic reasons as well as scientific and ecological purposes.

In 2002 a field survey was initiated in France in order to assess the overall contamination of 25 apiaries with pesticide residues, and whether the contaminants came from agricultural or beekeeping uses.[8] Only results on pesticide residues in beeswax are presented in this paper.

2 MATERIALS AND METHODS

Surveyed apiaries were distributed at five sites located in continental France (Fig. 1). At each site, five apiaries were chosen. In each apiary, five colonies randomly selected (i.e. 125 colonies in total) were sampled for beeswax once a year in October/November 2002 and 2003. In each hive surveyed, one frame was randomly selected for sampling and a lump of approximately 15 g of wax was cut off on a single area. Therefore, the sample included wax produced by honey bees and foundation wax. Foundation wax is the base of combs on which bees build cells. In this study, foundation wax has never been sampled on its own. The sampled area was selected with regard to its cleanliness whatever its colour, and cells had to be empty of any content (honey, beebread or larvae). Each sample was transported in a plastic container, then stored at $-20\,^{\circ}C$ until analysis. When brought back to the laboratory, all samples from the same apiary (i.e. five hives) were combined. Because of climatic conditions, it was not possible to sample beeswax in three apiaries. Chemical analyses were therefore conducted on the 47 pooled samples.

* Correspondence to: Marie-Pierre Chauzat, AFSSA, Les Templiers, 105 route des Chappes, BP 111-F-06 902 Sophia-Antipolis Cedex, France
E-mail: mp.chauzat@afssa.fr
(*Received 1 March 2006; revised version received 15 December 2006; accepted 21 December 2006*)
Published online 19 September 2007; DOI: 10.1002/ps.1451

 Pest Manag Sci 1526–498X/2007/$30.00

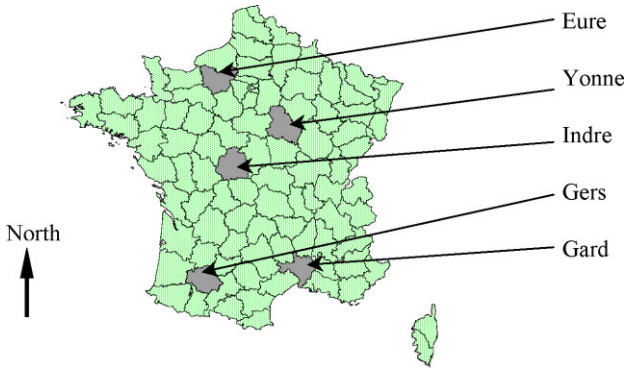

Figure 1. Location and names of the French sites surveyed.

Professional and non-professional beekeepers were asked to maintain their usual apicultural methods during the study. However, for practical reasons, they had been asked to keep all the surveyed colonies in the same location all year long (no migratory beekeeping).

2.1 Chemical analyses

Analyses were performed in the GIRPA laboratory (Groupement Interrégional sur les Recherches des Produits Agropharmaceutiques, Angers, France). Beeswax samples (2 g) were placed in 40 mL hexane. Samples were completely dissolved by ultrasound shaking and gentle heating (40 °C). Tubes were placed into liquid nitrogen for 2 min and then immediately centrifuged for 15 min (1424 × *g*). The supernatant fraction was collected and evaporated in a rotary evaporator (40 °C) until approximately 6 mL remained. To this residue was added 6 mL hexane and then 20 mL of hexane + acetonitrile (1 + 9, by volume). The solution was transferred into a separating funnel, vigorously shaken and subsequently allowed to separate for 20 min. The acetonitrile phase was collected, and the hexane fraction was extracted a second time with a further 20 mL of the acetronitrile/hexane mixture. The acetonitrile phases were pooled together and concentrated to 2 mL on a rotary evaporator. The extract was then ready for clean up.

Several C 18 Mega Bond Elut cartridges (1 g, 6 mL) were conditioned by successive elutions of 6 mL methanol, 6 mL water and 6 mL acetonitrile. The extract was percolated through the cartridge, which was then eluted with 15 mL of acetonitrile + water (5 + 1, by volume) and 20 mL acetonitrile added to the aliquot. The solution was dried in a rotary evaporator (40 °C) with 50 µL *n*-dodecane. Finally, the residue was dissolved in 1 mL ethyl acetate.

Chromatographic multiresidue analysis was performed with a 1200 triple-quadruple GC/MS/MS system (Varian Scientific Equipment, Palo Alto, CA). The chromatograph was fitted with a 30 m × 0.25 mm × 0.25 µm CP Sil 8 CB/MS column from Varian. The oven temperature programme consisted of 1 min at 60 °C, an increase of 20 °C min^{-1} up to 300 °C and 19.5 min at 300 °C. The carrier gas flow was kept constant at 1 mL min^{-1}. Injection (2 µL) was performed with a 1079 injector (SPI mode). The temperature programme consisted of 0.5 min at 60 °C, an increase of 200 °C min^{-1} up to 250 °C and 10 min at this last temperature. Conditions of detection were as follows: mode of ionisation was electronic impact, detector temperature 40 °C, transfer line temperature 300 °C, source temperature 300 °C, pressure pulse 3.3 Pa and electron multiplier voltage 70 eV.

Residues of 18 contaminants were searched for. Pesticide class, purpose of use and status (legal/withdrawn) for plant treatment in France in 2003 are detailed in Table 1. Pesticides were chosen because of their high toxicity towards honey bees and because of their frequent uses in the field.[9,10] Among the 18 active substances, 16 were insecticides or acaricides, and two were fungicides (Table 1). The use of 16 active substances from the list was legally authorised in 2003. Two pesticides were banned for plant protection use (coumaphos and lindane). The only commercial preparation containing coumaphos allowed for varroa control was not sold in France in 2002 and 2003.

Limits of detection (LOD) were 5.0 µg kg^{-1} for all materials. Limits of quantification (LOQ) were 20.0 µg kg^{-1} for parathion-methyl and deltamethrin, and 10.0 µg kg^{-1} for all the other compounds.

2.2 Statistical analysis

Percentages of polluted wax samples were calculated by dividing the number of positive samples (samples where the selected compound was detected) by the total number of samples analysed for this compound and multiplying by 100. The average content was calculated using the arithmetic mean when values were between LODs and LOQs.

Statistical tests were conducted on frequencies, not on 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 makes it possible to estimate the probability that an event could occur when the explicative variable is known: $P(Y|X_1, X_2, \ldots, X_p)$. The maximum likelihood ratio and the type III tests were used to estimate the coefficient of the model. When these two indicators showed 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's exact test was performed. Unless otherwise stated, the significance threshold was 5%. All tests were performed using SAS software (SAS system for Windows, V8).

3 RESULTS

3.1 Frequency and average content of pesticide residues

A total of 47 beeswax samples were analysed. Residues of 14 active substances were found in 33 samples of

Table 1. Characteristics of the surveyed pesticides: chemical class, purpose of use, legal status for agricultural use in 2003, limits of detection and limits of quantification

Pesticide	Pesticide class	Purpose of use[a]	Status in 2003[b]	LOD[c]	LOQ[d]
Azinphos-methyl	Organophosphate	I	A	5.0	10.0
Chlorpyrifos	Organophosphate	I	A	5.0	10.0
Coumaphos	Organophosphate	I, A	B	5.0	10.0
Cyfluthrin	Pyrethroid	I	A	5.0	10.0
Cypermethrin	Pyrethroid	I	A	5.0	10.0
Deltamethrin	Pyrethroid	I	A	5.0	20.0
Endosulfan	Organochlorine	I	A	5.0	10.0
Fenitrothion	Organophosphate	I	A	5.0	10.0
Fenthion	Organophosphate	I	A	5.0	10.0
Lindane	Organochlorine	I	B	5.0	10.0
Malathion	Organophosphate	I	A	5.0	10.0
Methidathion	Organophosphate	I	A	5.0	10.0
Mevinphos	Organophosphate	I	A	5.0	10.0
Parathion	Organophosphate	I	A	5.0	10.0
Parathion-methyl	Organophosphate	I	A	5.0	20.0
Tau-fluvalinate	Pyrethroid	I, A	A	5.0	10.0
Procymidone	Dicarboximide	F	A	5.0	10.0
Vinclozolin	Dicarboximide	F	A	5.0	10.0

[a] A: acaricide; F: fungicide; I: insecticide.
[b] A: authorized; B: banned.
[c] Limits of detection ($\mu g\,kg^{-1}$).
[d] Limits of quantification ($\mu g\,kg^{-1}$).

Table 2. Pesticide residues in beeswax samples: pollution frequencies and contents[a]

Pesticide	Number of positive samples	Frequency (%)	Residue concentrations min. ($\mu g\,kg^{-1}$)	Residue concentrations max. ($\mu g\,kg^{-1}$)	Average concentration ($\mu g\,kg^{-1}$)
Azinphos-methyl	2	10.0	75.2	817.0	446.1
Chlorpyrifos	3	7.3	7.1	19.0	14.9
Coumaphos	24	52.2	>LOD	4 112.6	792.6
Cyfluthrin	5	12.2	5.9	31.9	20.1
Cypermethrin	7	21.9	14.2	76.3	36.3
Deltamethrin	1	2.4	14.7	14.7	14.7
Endosulfan	11	23.4	14.7	243.1	88.8
Fenitrothion	1	2.1	511.0	511.0	511.0
Fenthion	0	0.0	ND	ND	ND
Lindane	2	4.3	5.3	32.2	18.8
Malathion	4	8.5	10.2	18.1	15.1
Methidathion	0	0.0	ND	ND	ND
Mevinphos	0	0.0	ND	ND	ND
Parathion	1	2.1	99.0	99.0	99.0
Parathion-methyl	0	0.0	ND	ND	ND
Tau-fluvalinate	13	61.9	15	422	196.4
Procymidone	1	2.1	27.7	27.7	27.7
Vinclozolin	1	2.1	21.5	21.5	21.5

[a] ND: not detected.

wax. The most frequent residues identified were tau-fluvalinate (in 61.9% of samples), coumaphos (52.2%) and endosulfan (23.4%). Frequencies of other residues ranged from 2.1 to 21.9%.

Coumaphos had the highest average content ($792.6\,\mu g\,kg^{-1}$), with a maximum value of $4112.6\,\mu g\,kg^{-1}$. Fenitrothion residues were found in the second average highest quantities, with $511.0\,\mu g\,kg^{-1}$ found in a single sample. It is worth noting that the banned substance lindane was quantified in two samples at an average content of $18.8\,\mu g\,kg^{-1}$ (Table 2). Only 14 samples (29.8%) were found to be free of any searched pesticides (Fig. 2). One residue was found in 34.0% of samples. Residues of more than one compound (up to eight different molecules) were found in the remaining 36.2% of samples.

3.2 Comparison between sampling years

For statistical analysis, pesticides were grouped according to their pesticide class (Table 1). Contamination frequencies in beeswax are graphed in Fig. 3

Pest Manag Sci **63**:1100–1106 (2007)
DOI: 10.1002/ps

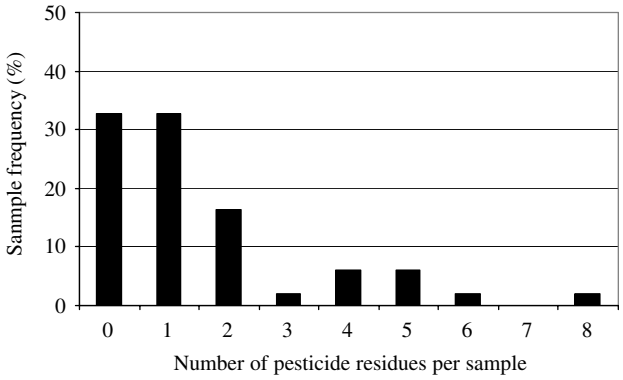

Figure 2. Frequencies of numbers of different pesticides in beeswax samples.

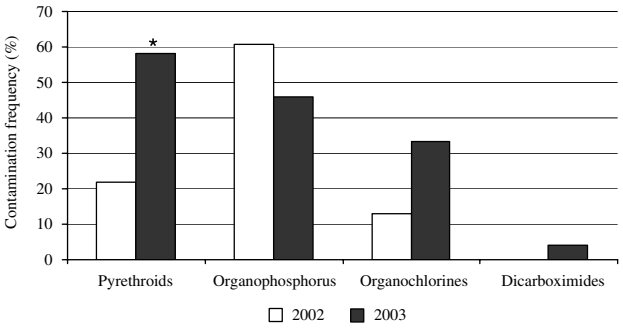

Figure 3. Frequency of contamination of wax samples by various pesticide families. Samples were considered positive when residues were superior to the LOD. * indicates significant difference from the previous year ($P < 0.05$).

for each pesticide class. In 2002, organophosphate residues were the most frequently identified substances found in wax samples: ca 61% of collected samples contained organophosphate residues, compared with ca 46% in 2003. In 2003, pyrethroid residues were the most frequent (present in ca 58% of samples), in contrast to 2002, when only ca 22% of samples were positive in pyrethroids. This increase in frequency was the only statistically significant yearly variation ($\chi^2 = 6.5$, 1 df, $P < 0.01$).

4 DISCUSSION

4.1 Beeswax contamination and apicultural practices

In the present study, coumaphos residues had the second highest frequency of wax contamination (52.2%) and the highest average content (792.6 $\mu g\,kg^{-1}$). These figures are comparable with those collected from literature references. Coumaphos residues can originate from varroa treatments and from foundation wax. In Germany it was demonstrated in 1999 that 62.5% of German foundation wax samples and 20% of foundation wax from international samples contained coumaphos.[11] Numerous surveys have been run in European countries[12,13] and in the USA[14] to assess the level of coumaphos contamination in beeswax.

If acaricide residues are present in beeswax, it is legitimate to wonder whether they could act as an effective treatment against varroa. However, it has been shown that coumaphos residues would have to be far more concentrated than the levels the authors observed to have any impact on varroa populations: honey bee cocoons provide a very effective barrier that prevents mortality of female mites.[15] Conclusions were similar with regards to fluvalinate concentrations observed in brood combs.[15]

Coumaphos and tau-fluvalinate are administered in different ways to honey bees, by either powder or strips. The amount of acaricide finally distributed throughout the colony depends on the mode of application, the quantity applied to hives, its solubility in wax and its stability. When strips are used, the quantity of acaricide within the hive depends on the activity of honey bees and on the duration of the application. Usually, acaricide residues in hives are lower when strips are used rather than powder.[11]

4.2 Pesticide residues and environmental pollution

Residues of tau-fluvalinate were the most frequently found of all pesticides (in 61.9% of all samples). The frequency and quantities of this compound found in the present study were consistent with the figures present in the literature (Tables 3 and 4). For example, in a survey conducted in Canada in 2003, fluvalinate residues were found in 38.5 and 91.7% of wax samples from honey supers and from brood chambers respectively. This pesticide is used for both agricultural and varroa control purposes, and it is therefore difficult to state the origin of contamination with any certainty.

In the present study it has been shown that several products used for plant protection are frequently found in beeswax. Very few references in the literature report this kind of contamination. Interestingly, Kubik *et al.*[16] have studied vinclozolin in bee bread. They found far higher levels of residues of this compound in bee bread than in pollen. One possible explanation indicated by the authors could be the conjugation of the pesticide to pollen grains. This chemical bond would prevent the pesticide being extracted with the solvent at the early steps of analysis. During fermentation of bee bread, this bond could be broken and the pesticides released.[16] As bee bread is stored in beeswax cells, this process could provide a possible route for transfer of compounds between these two matrices.

Although a high variation in contamination frequencies according to the year of sampling was observed, only the increase in pyrethroid occurrence from 2002 to 2003 was statistically significant. This discrepancy has to be put in perspective with the sampling mode that was probably not representative of hive contamination in particular but representative of an overall apiary contamination. Various authors have already mentioned the difficulty of evaluating contaminant distribution within hives, particularly in beeswax.[1,17]

Table 3. Pesticide contents in beeswax as reported in the literature[a]

Pesticide	Contents min.–max.	LOD min.–max.	Protocol	Reference
Coumaphos	3.8	0.4–1.0	Experiment	3
	0.7–67	0.5	Experiment	15
	0.06–82	0.01	Experiment	17
	0.5–3.5	0.5	Foundation wax	11
	1.2–2.6	0.4–1.0	Foundation wax	3
	1.5–4	0.5	Foundation wax	4
	0.2	0.01	Foundation wax	17
	0.08–2.8	0.005	Survey in Greece	22
	0.7–1.3	0.2–0.3	Survey in Switzerland	1
	3–8	NR	Survey in USA	14
	0.8–2.5	NR	Survey in Italy	23
	0.27–0.38	0.015	Foundation wax	2
	0.2–5.8	NR	Survey in France	13
Lindane	0.2	0.05	Foundation wax	4
	0.042–0.29	0.001	Foundation wax	2
Malathion	0.05–6.0	0.0005	Survey in Greece	22
Tau-fluvalinate	1.8	0.4–1	Experiment	3
	0.8–84	0.05	Experiment	15
	0.5–3.5	0.5	Foundation wax	11
	0.8–8	0.5	Foundation wax	4
	0.6–0.8	0.4–1	Foundation wax	3
	1.1–2.4	NR	Survey in Canada	14
	2.6	NR	Survey in USA	14
	0.1–1.6	NR	Survey in Italy	12
	0.1–3.6	NR	Survey in France	13
Vinclozolin	ND	0.5	Foundation wax	4

[a] Contents and LOD are expressed in mg kg^{-1}. ND: not detected; NR: not reported.

Table 4. Acute toxicity of pesticides for brood and adult bees

Insecticide	Brood LD_{50}[a] (µg $larva^{-1}$)	Adult LD_{50}[a] (µg bee^{-1})	Mode of administration[b]	Reference
Azinphos-methyl	NA	NT	NR	9
		0.43	U	24
Chlorpyrifos	NA	0.11	U	9
Coumaphos	NA	3–6	O	25
Cypermethrin	0.066	0.06	U	10
Deltamethrin	NA	0.7	O	26
Endosulfan	28.142	21.79	U	10
Fenitrothion	NA	0.28	U	9
Fenthion	NA	0.30	U	9
Malathion	0.736	0.73	U	10
Methidathion	0.274	0.24	U	10
Mevinphos	0.441	0.31	U	10
Parathion	NA	0.07–0.10	T	27
		0.09–0.13	O	
Parathion-methyl	NA	0.29	U	9
Tau-fluvalinate	NA	65.85	U	24

[a] NT: non toxic; NA: not available in the literature.
[b] For adults. T: topical; O: oral; U: unknown; NR: not relevant.

4.3 Toxicity to bees

All acaricides have been tested for honey bee toxicity prior to registration and have been found non-toxic when used at recommended doses. However, the possibility exists that synergistic effects between the different pesticides might lead to a toxic effect on bees.[1] Moreover, even with a single compound, possible long-term toxicity must be considered, as honey bees are in contact with beeswax throughout their lives. Obviously, larvae that are reared in cells are in closer contact with residues contained in the wax. Indeed, acaricide residues have already been found in larvae.[17] The possible impacts of pesticides and pesticide cocktails to larval bees needs

Pest Manag Sci **63**:1100–1106 (2007)
DOI: 10.1002/ps

to be further explored. A recent publication on a method for larva rearing could facilitate further studies that may shed more light on this particular topic.[18]

Adult honey bees are exposed to residues contained in wax mainly by contact as they walk on frames, process nectar into honey, feed and take care of the brood. Combs are continuously modified so that the colony models its dynamics on the extent of honey flow. Size of cells are adapted (i.e. male cells), emptied of their content (bee bread, for example) and cleaned. Honey bees do not actually eat beeswax, but the process involves chewing beeswax, thus exposing individuals to pesticide residues.

The hazard assessment of pesticides to honey bees is commonly determined through the use of laboratory studies (median lethal dose LD_{50}). Doses of pesticide residues found in this survey were lower than the LD_{50} values of the pesticides investigated. However, such small doses may have sublethal effects on honey bees, as has been demonstrated in a number of cases. For example, the negative effect of coumaphos and fluvalinate on queen rearing has been studied.[19,20] In the USA, queen failures associated with sublethal toxicity to honey bees were also observed in the field when high levels of coumaphos were detected.[14]

Beekeepers should consider rotating older combs out of their operation as a way to eliminate pesticide residues from the hives. As bees that have fed upon stored contaminated materials produce polluted honey and wax,[17] this precaution would avoid the contamination of hive products. Van Buren *et al*.[21] confirmed that newly produced wax contained an average of 520 $\mu g\,kg^{-1}$ of coumaphos 6 months after colony treatment.

The conversion of nectar into honey involves physical and chemical changes. During the honey maturing process, bees reduce nectar water content, while the chemical changes are consecutive to enzyme addition from honey bee glands. All these steps are necessary to make the honey into a compound with a long life. Pesticide transmission rate from nectar to wax is high when the nectar stored has a relatively high water content. The diffusion rate decreases with honey maturation process. Rape honey has been found to be too viscous to be cleaned by the diffusion of pesticide residues into beeswax (Wallner K, personal communication, 2005). This result, added to other experiments, suggests that, under certain conditions, wax seems to act as a sink for hydrophobic substances such as coumaphos.[17] This would represent a good opportunity for beekeepers to prevent the build-up of pesticide residues in the hive environment by simply removing old combs.

Once removed from hives, technical options for removing pesticides from wax are limited. Concentration does not decrease during the process of producing new wax from old melted combs: coumaphos contents did not decrease in wax after 2 hours at 140 °C.[1] Only complete destruction by burning wax can destroy stored contaminants.[11] Therefore, the authors strongly encourage beekeepers to make foundation wax from their own virgin wax.

5 CONCLUSIONS

This study has shown the high contamination of beeswax by pesticide residues coming from varroa treatments on the one hand and from plant protection on the other. The impact of this contamination on honey bees is still difficult to assess. In Europe, acaricide residues in beeswax are not regulated. Beekeepers and various industries benefit from the healthy and pure image that bee products present to the public. To protect this image, and also to protect bee population health, it may be important to minimise or eliminate pesticide residues from bee products. Rotating older combs out of hives provides an opportunity to reduce the amount of pesticides inside colonies, provided that commercial foundation wax does not contain any residues.

ACKNOWLEDGEMENTS

The authors wish to thank Zeggane S, Aubert TLF, the referees who read this paper and Baragatti M for her assistance with statistics. Many thanks also to all the apiarists who volunteered their hives and their time for this survey, to the veterinary services of French sites for assisting during the study and to the GIRPA laboratory. This survey has been partly funded by the European Community through the FEOGA project (CE 1221/97).

REFERENCES

1 Bogdanov S, Kilchenmann V and Imdorf A, Acaricide residues in some bee products. *J Apic Res* **37**:57–67 (1998).
2 Jimenez JJ, Bernal JL, del Nozal MJ and Martin MT, Residues of organic contaminants in beeswax. *Eur J Lipid Sci Technol* **107**:896–902 (2005).
3 Bogdanov S and Kilchenmann V, Acaricide residues in beeswax: long-term studies in Switzerland. *Apidologie* **26**:319–321 (1995).
4 Wallner K, The actual beeswax quality in foundations from the market. *Apidologie* **28**:168–170 (1997).
5 Lodesani M, Pellacani A, Bergomi S, Carpana E, Rabitti T and Lasagni P, Residue determination for some products used against varroa infestation in bees. *Apidologie* **23**:257–272 (1992).
6 Bogdanov S, Contaminants of bee products. *Apidologie* **37**:1–18 (2006).
7 Leita L, Muhlbachova G, Cesco S, Barbattini R and Mondini C, Investigation of the use of honey bees and honey bee products to assess heavy metals contamination. *Environ Monitor Assess* **43**:1–9 (1996).
8 Chauzat MP, Faucon JP, Martel AC, Lachaize J, Cougoule N and Aubert M, A survey on pesticide residues in pollen loads collected by honey-bees (*Apis mellifera*) in France. *J Econ Entomol* **99**:253–262 (2006).
9 Ghini S, Fernandez M, Pico Y, Marin R, Fini F, Manes J, *et al*, Occurrence and distribution of pesticides in the province of Bologna, Italy, using honeybees as bioindicators. *Arch Environ Contam Toxicol* **47**:479–488 (2004).

10 Atkins EL and Kellum D, Comparative morphogenic and toxicity studies on the effect of pesticides on honeybee brood. *J Apic Res* **25**:242–255 (1986).
11 Wallner K, Varroacides and their residues in bee products. *Apidologie* **30**:235–248 (1999).
12 Lodesani M, Costa C, Bigliardi M and Colombo R, Acaricide residues in bee wax and organic beekeeping. *Apiacta* **38**:31–33 (2003).
13 AFSSA Sophia-Antipolis, Les contaminations chimiques des cires. *La Santé de l'Abeille* **187**:38–42 (2002).
14 Nasr ME and Wallner K, Miticides residues in honey and wax in North America. *Am Bee J* **143**:322 (2003).
15 Fries I, Wallner K and Rosenkranz P, Effects on *Varroa jacobsoni* from acaricides in beeswax. *J Apic Res* **37**:85–90 (1998).
16 Kubik M, Nowacki J, Pidek A, Warakomska Z, Michalczuk L and Goszczynski W, Pesticides residues in bee products collected from cherry trees protected during blooming period with contact and systemic fungicides. *Apidologie* **30**:521–532 (1999).
17 Tremolada P, Bernardinelli I, Colombo M, Spreafico M and Vighi M, Coumaphos distribution in the hive ecosystem: case study for modeling applications. *Ecotoxicology* **13**:589–601 (2004).
18 Aupinel P, Fortini D, Dufour H, Tasei JN, Michaud B, Odoux JF, *et al*, Improvement of articial feeding in a standard *in vitro* method for rearing *Apis mellifera* larvae. *Bull Insectol* **58**:107–111 (2005).
19 Haarmann T, Spivak M, Weaver D, Weaver B and Glenn T, Effects of fluvalinate and coumaphos on queen honey bees (Hymenoptera: Apidae) in two commercial queen rearing operations. *J Econ Entomol* **95**:28–35 (2002).
20 Pettis JS, Wilson WT, Shimanuki H and Teel PD, Fluvalinate treatment of queen and worker honey bees (*Apis mellifera* L.) and effects on subsequent mortality, queen acceptance and supercedure. *Apidologie* **22**:1–7 (1991).
21 Van Buren NWM, Mariën J, Velthuis HHW and Oudejans RCHM, Residues in beeswax and honey of Perizin, an acaricide to combat the mite *Varroa jacobsoni* Oudemans (Acari: Mesostigmata). *Environ Entomol* **21**:860–865 (1992).
22 Thrasyvoulou AT and Pappas N, Contamination of honey and wax with malathion and coumaphos used against the varroa mite. *J Apic Res* **27**:55–61 (1988).
23 Westcott LC and Winston ML, Chemical acaricides in *Apis mellifera* (Hymenoptera: Apidae) colonies: do they cause nonlethal effects? *Can Entomol* **131**:363–371 (1999).
24 Reducing pesticide hazards to honey bees: mortality prediction techniques and integrated management strategies. *University of California leaflet 2883*, pp. 1–22 (1981).
25 Van Buren NWM, Mariën AGH, Oudejans RCHM and Velthuis HHW, Perizin, an acaricide to combat the mite *Varroa jacobsoni*: its distribution in and influence on the honeybee *Apis mellifera*. *Physiol Entomol* **17**:288–296 (1992).
26 Decourtye A, Devillers J, Cluzeau S, Charreton M and PhamDelegue MH, Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. *Ecotoxicol Environ Safety* **57**:410–419 (2004).
27 Murray A, Acute and residual toxicity of a new pyrethroid insecticide, WL85871, to honey-bees. *Bull Environ Contam Toxicol* **34**:560–564 (1985).

Pest Manag Sci **63**:1100–1106 (2007)
DOI: 10.1002/ps