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Original article

Predicting pesticide fate in the hive (part 1): experimentally determined τ-fluvalinate residues in bees, honey and wax

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Abstract – τ-Fluvalinate residues in bees, honey and wax were measured in two experimental hives treated with Apistan to test a multi-compartmental predictive model. Pesticide residues were monitored for 30 days after treatment in bees and for up to 180 days in honey and wax. Concentrations ranged between 14 and 160 ng g^{-1} f.w. in bees and between 98 and 1630 ng g^{-1} in wax, while no residues were detected above the analytical limit (2.5 ng g^{-1}) in honey. τ-Fluvalinate residues are discussed in the context of a survey of data from the literature on other pesticides (bromopropylate, coumaphos, malathion and amitraz). This data review shows that residues of the same compound exhibit extremely high variability within the same matrix. This finding underlines the importance of developing predictive tools for both post-treatment analysis and a priori evaluation of the possible contamination effects of pesticides depending on the mode of application.

fluvalinate / pesticide residue / wax contamination / honey contamination / bee health

1. INTRODUCTION

Pesticides can reach a beehive from outside (Haouar et al. 1990; Chauzat et al. 2006) or from inside by chemical treatment against honeybee parasites (Rice et al. 2004). The mite *Varroa destructor* is one of the most harmful and widely distributed parasites of honeybees (*Apis mellifera ligustica*). A variety of defensive treatments have been developed using synthetic acaricides (e.g. coumaphos, τ-fluvalinate, malathion and bromopropylate) or vegetable and/or essential oils, especially thymol (Rice et al. 2004). In addition to problems of toxicity to bees (Melathopoulos et al. 2000; Decourtye et al. 2005; Halm et al. 2006), pesticide residues in hive matrices and in human-consumed bee products (honey, propolis, royal jelly) pose significant concerns. Many analytical methods have been developed for monitoring purposes (Menkissoglu-Spiroudi et al. 2000; Fernandez et al. 2002; Russo and Neri 2002; Rial-Otero et al. 2007), and a large amount of data has been obtained, beginning with the work of Thrasyvoulou et al. (1985) and continuing through that of Lodesani et al. (2008). Reviews published by Wallner (1999), Bogdanov (2006) and Johnson et al. (2010) offer a broad view of the problem of pesticide contamina-

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tion of bee products. Fat-soluble and stable pesticides, such as bromopropylate, coumaphos and τ-fluvalinate, are the most abundant residues in wax (Wallner 1999), accumulating year by year (Bogdnov 2006). Fat-soluble and volatile compounds, such as essential oils, and unstable pesticides, such as amitraz, do not accumulate as much in the wax matrix (Wallner 1999). On the other hand, water-soluble chemicals, such as formic acid, oxalic acid and cymiazole, do not accumulate in wax. However, they are found in honey, producing an unwanted taste (Wallner 1999). Residues of fat-soluble and stable pesticides have been found in surveys in many countries: Greece (Thrasyvoulou and Pappas 1988), Italy (Lodesani et al. 1992, 2008; Russo and Neri 2002), Switzerland (Bogdanov et al. 1998, 2003), France (Martel et al. 2007; Chauzat and Faucon 2007), Germany (Wallner 1999), Spain (Fernandez-Muiño et al. 1995, 1997; García et al. 1996; Jiménez et al. 2005), Portugal (Rial-Otero et al. 2007), North America (Nasr and Wallner 2003; Mullin et al. 2010) and Saudi Arabia (Kamel and Al-Ghamdi 2006). Experiments designed to assess the persistence, fate and metabolism of various pesticide compounds have been performed over periods of up to 2 years using experimental hives (van Buren et al. 1992; Bogdanov and Kilchenmann 1995; Fries et al. 1998; Tsigouri et al. 2001, 2004; Waliszewski et al. 2003; Tremolada et al. 2004; Martel et al. 2007).

Among synthetic acaricides, τ-fluvalinate has been one of the most frequently used anti-*Varroa* pesticides. This compound is available in various forms, including Apistan (plastic strips placed into the hive for a typical period of 4 weeks; Cabras et al. 1997), Klartan and Mavrik (water suspensions; Wallner 1999; Tsigouri et al. 2004). Problems of resistance in *V. destructor* (Martin 2004) and pesticide residues in honey and wax (Waliszewski et al. 2003) have reduced the use of τ-fluvalinate. For example, since the appearance of τ-fluvalinate-resistant mites in Switzerland during the 1990s, its use in that country, and consequently, its residues in commercial wax have decreased. However, another 20 years have been estimated to be necessary before τ-fluvalinate residues fully disappear from beeswax (Bogdanov 2006). Fluvalinate resists the melting and purification operations used during the production of new foundation wax (Bogdanov et al. 1998), transferring the contamination to hives that have not been treated with τ-fluvalinate or furnishing additional residues to treated hives. In foundation wax produced in Germany, τ-fluvalinate is often detectable within the range 500–3,500 ng g^{-1} (Wallner 1999). The rate of τ-fluvalinate accumulation in wax has been estimated to be approximately 500 ng g^{-1} $year^{-1}$ (Nasr and Wallner 2003). τ-Fluvalinate residues have also been found in propolis at higher concentrations than in wax (Bogdanov et al. 1998). Even though its use is no longer common, this compound should continue to be carefully monitored as a case study for understanding the processes that determine the fate of extraneous molecules in the hive system. The behaviour of a chemical in the hive ecosystem depends on many factors: its chemical and physical properties, the means by which it enters the hive and the biological and physical characteristics of the specific environment (Mackay 2001). The fate of a compound in the hive is determined by several time-dependent processes: uptake, distribution, biotransformation, volatilisation, diffusion within matrices, phase partitioning, advection from the hive by air ventilation and bee turnover and product collection by beekeepers. The relative importance of these processes is essential in determining the fate of the pesticide in the hive and therefore, the contamination effects in bees and their products. Mathematical modelling of these processes would help to elucidate the behaviours of the different chemicals over time. However, model development requires a foundation of experimental data. In this paper, we present a time series of τ-fluvalinate concentrations in bees, honey and wax measured in two experimental hives for up to 6 months after treatment with Apistan. These experiments are intended to provide homogeneous time series of τ-fluvalinate contamination data in multiple matrices. These data will then

be used for model development and validation (part 2). The overall goal of this study is to experimentally validate a multi-compartmental hive model able to predict the concentrations of organic contaminants whether they enter the hive through direct treatment or from the outside.

2. MATERIALS AND METHODS

2.1. Hive treatment and sample collection

Two experimental hives (50×50 cm and 33 cm high), situated next to each other and equipped with ten honeycombs (47×30 cm) each, were treated with Apistan on 17 August 2000 after removing supplementary honeycombs. The number of hives was limited, but it was considered sufficient for evaluating the between-hive variability. During the treatment period, the size of each colony was estimated to be 40,000–50,000 adult bees. The commercial product Apistan contains τ-fluvalinate as its active ingredient. The pesticide is contained on PVC strips, each with 0.8 g of active ingredient. Two strips were hung in each hive (one between the third and fourth honeycomb and one between the seventh and eighth honeycomb) for 1 month (strips were removed on 16 September 2000). A total of 1.6 g of active ingredient was present in each hive. The pesticide enters the bees mainly by contact with the strips, providing long-lasting protection against the parasite, even when brood is present (Greatti et al. 1992).

Samples of adult bees, honey and wax were taken before treatment and up to 180 days after treatment (sampling schedule shown in Table I). Honey was chosen as representative matrix of bee foods because of its importance as foodstuff. Each honey or wax sample was obtained by homogenising multiple sub-samples taken from different points in different honeycombs within the nest. Bee samples (30 animals each) were obtained by picking up four to five animals from different points in the hive. All samples were immediately frozen for storage (−20°C). Homogenised samples were designed to increase the representativeness of the analytical results.

2.2. Chemical analyses

2.2.1. Honey extraction

Two 10-g aliquots were taken from each sample and extracted separately on two C18 SPE cartridges (containing 1 g of phase from Altech) in order to optimise the extraction times. Each aliquot was diluted with 25 mL each of distilled water/acetone (1:1) in a beaker. Once homogenised, each honey solution was extracted using C18 SPE cartridges. C18 cartridges were previously washed with 5 mL of *n*-hexane and activated with 5 mL of methanol and 5 mL of distilled water. Samples were loaded onto the columns and 1 mL of *n*-hexane was used to rinse the beaker. Then the columns were air dried for 30 min and eluted with 10 mL of dichloromethane. The two dichloromethane fractions coming from the extractions of the two honey aliquots were mixed, concentrated by Rotor evaporator and dried under a gentle nitrogen stream. The extract was re-suspended in *n*-hexane to a final volume of 0.5 mL for GC-MS quantification.

2.2.2. Bee extraction

Thirty bees were homogenised with 8 g of anhydrous Na_2SO_4 and extracted with 50 mL of *n*-hexane in a Soxtec apparatus (VELP, Italy) for 4 h. The extracts were reduced by Rotor evaporator to a volume of 2 mL, cooled in a freezer for 1.5 h and centrifuged for 15 min at 10,000 rpm at −5°C. The supernatant was transferred to a new centrifuge tube and the centrifugation repeated. The final supernatant (1 mL) was ready for the Florisil purification described below.

2.2.3. Wax extraction

Wax samples (1.5 g) were dissolved in 10 mL of *n*-hexane using an ultrasonic apparatus for 45 min at 40°C. Wax impurities were discarded, and the wax weight was corrected. The *n*-hexane solution was cooled in a freezer for 1.5 h and centrifuged for 18 min at 10,000 rpm at −5°C. The supernatant was concentrated to a volume of 1 mL using a Rotor evaporator and then transferred to a new centrifuge tube. The centrifuge procedure was repeated twice. The final supernatant

Table I. τ-Fluvalinate concentrations in bees, honey and wax from the two experimental hives before treatment (T_0) and at different times after treatment (from T_1 to T_{12}).

Time	Days	τ-Fluvalinate concentration (ng g^{-1})					
		Bees		Honey		Wax	
		Hive 1	Hive 4	Hive 1	Hive 4	Hive 1	Hive 4
T_0	0	<10	<10	<2.5	<2.5	210	170
T_1	0.8	82	24	–	–	–	–
T_2	1	160	79	<2.5	<2.5	230	160
T_3	2	100	68	–	–	–	–
T_4	4	160	130	<2.5	<2.5	98	370
T_5	7	58	14	–	–	–	–
T_6	10	64	25	<2.5	<2.5	114	220
T_7	15	62	66	–	–	–	–
T_8	30	46	59	<2.5	<2.5	280	650
T_9	30.25	50	66	<2.5	<2.5	570	670
T_{10}	60	–	–	<2.5	<2.5	904	1,630
T_{11}	120	–	–	<2.5	<2.5	1,130	–
T_{12}	180	–	–	<2.5	<2.5	–	1,540

Strips containing the pesticide were removed between T_8 and T_9

(1 mL) was ready for the Florisil purification described below.

2.2.4. Purification of bee and wax extracts

The final supernatant from each bee or wax extraction (1 mL) was loaded onto a Florisil (60/100 mesh) column (1.5 g Florisil+0.5 g anhydrous Na_2SO_4 in a 6-mm i.d. glass column). A first eluting fraction of 20 mL of *n*-hexane was discarded, while a second eluting fraction of 10 mL of *n*-hexane/acetone (1:1) was collected, concentrated by Rotor evaporator and dried under a gentle nitrogen stream. The dried purified extract was re-suspended in 0.5 mL of *n*-hexane for GC-MS quantification.

2.2.5. GC-MS analyses

A Hewlett Packard instrument (HP 5890 gas chromatograph and HP 5972 mass spectrometer) was used in selective ion monitoring mode. Quantitative analyses utilised a calibration curve based on an internal standard (permethrin) at τ-fluvalinate/permethrin ratios from 0.5:5 to 5:5. Control analyses were performed on parallel samples confirming the absence of detectable levels of permethrin. Monitored ions were 250 and 181 for τ-fluvalinate and 183 and 163 for permethrin. One-microlitre samples were injected in splitless mode. The carrier gas was helium with a flux of 1 mL min^{-1}. Chromatographic separation was performed on an HP 5 capillary column (30-m length, 0.25-mm i.d., 0.25-μm film thickness). Oven temperature was programmed at 60°C for 2 min followed by an increase from 60°C to 250°C at a rate of 10°C min^{-1} and then from 250°C to 280°C at a rate of 20°C min^{-1}, and finally, maintained at 280°C for 14 min. Chromatograms reporting τ-fluvalinate and permethrin pecks in bees, wax, honey and in the standard mixture are reported in Figure 1.

2.2.6. Quality assurance and quality control

Procedural blanks were run for each sample type, and negligible levels were found in all blank samples. Analytical procedures were checked for recovery and reproducibility. Recovery percentages were investi-

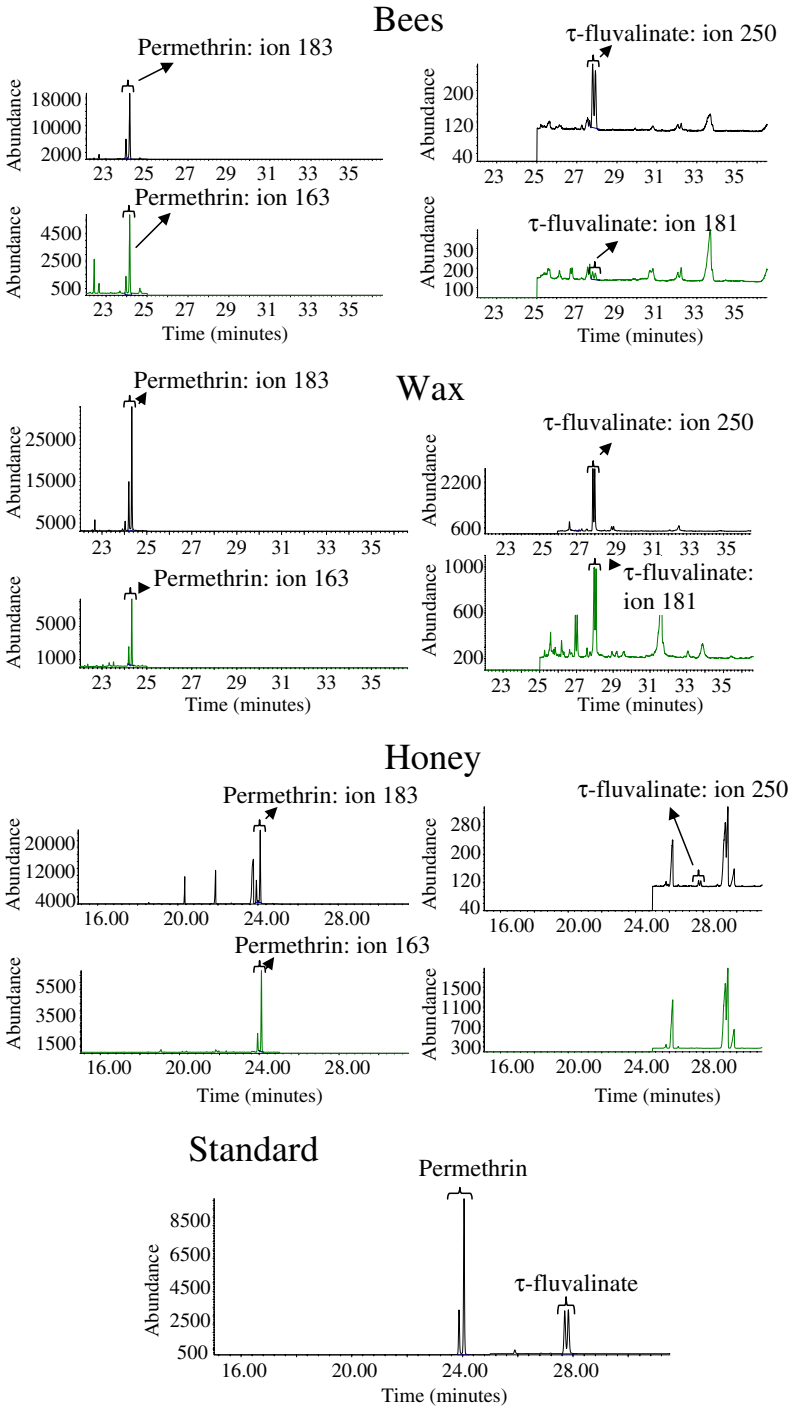

Figure 1. Chromatograms of the selected ions of τ-fluvalinate and permethrin in bees, wax and honey and chromatogram of the total ion current of the standard mixture containing 5 ng μL^{-1} of both permethrin and τ-fluvalinate. Pyrethroids contain chiral centres giving chromatographic double peaks (Feo et al. 2010). Reported chromatograms were 'Hive4-T3', 'Hive1-T12' and 'Hive4-T1' for bees wax and honey, respectively.

gated by spiking three sub-samples of each sample type with a known quantity of a τ-fluvalinate. Mean recovery percentages were 87%, 78% and 65% for honey, bees and wax, respectively. Reproducibility was calculated from replicated analyses on three sub-samples for each matrix, giving error rates of less than 10% for honey and bees and 20% for wax. Based on the recovery percentages, the results for the three matrices were corrected by factors of 1.15, 1.28 and 1.53 for honey, bees and wax, respectively. The limit of detection of τ-fluvalinate was 0.1 ng injected (signal-to-noise ratio 3:1). The limits of quantification of τ-fluvalinate in honey, bees and wax were 2.5, 10 and 35 ng g^{-1}, respectively (average amounts extracted from 20, 5 and 1.5 g of honey, bees and wax, respectively). Additional details and specific chromatograms can be asked to the authors.

3. RESULTS

Analytical results are reported in Table I. No detectable residues were present in honey due to the low water solubility of τ-fluvalinate (0.001 mg L^{-1}; Tomlin 1997) and the high *n*-octanol/water partition coefficient (Log K_{ow}=4.3; Tomlin 1997). Lodesani et al. (1992) and Bodganov et al. (1998) have previously reported low τ-fluvalinate concentrations in honey (<1–2 and <3–5 ng g^{-1}, respectively), confirming our results.

Residues in bees (Figure 2) were not detectable before treatment but increased until the fourth day after treatment with high variability among samples and then rapidly decreased from the seventh day onward. By the 30th day, residues in bees reached a nearly constant level (around 50 ng g^{-1} f.w.). The initially high variability of bee contamination levels is due to differences in activity among bees (Winston 1991). Individual bees differ in their probability of coming into contact with the pesticide and thus in their level of contamination.

Residues were present in wax before treatment due to previous treatments or contaminated material. The methods used to generate the new wax of foundation combs do not eliminate τ-fluvalinate residues (Lodesani et al. 2008). After treatment, τ-fluvalinate concentrations in wax slowly increased from the end of the first week until the 60th day and then maintained a near constant level through the 180th day.

The fate of the pesticide was evaluated quantitatively based on the mass balance data reported in Table II. Two different times were chosen: T_4 (4 days after treatment), when mean concentration in bees reached their maximum, and T_{10} (60 days after treatment), when mean concentrations in wax reached their maximum. At T_4, the total amount recovered in the monitored matrices was 0.8 mg, of which about 90% was present in bees. At T_{10}, the total amount recovered was about 2 mg, of which about 85% was present in wax. The maximum amount recovered was almost 2 mg of active ingredient, about 0.125% of the quantity of pesticide introduced in the hive (1.6 g). These results are consistent with those of Cabras et al. (1997), who found no appreciable differences in the τ-fluvalinate content of the PVC strips before and after treatment. These results suggest that τ-fluvalinate leaves the PVC strips very slowly and that the amount transferred to the hive matrices is limited compared to that introduced. Nevertheless, its capacity to persist within the hive matrices is high: the concentration in bees remains high after 30 days and that in wax, increases until the 60th day. Wax contamination appears to be the most important negative effect of this pesticide because of the nearly constant contamination levels even 150 days after the removal of the pesticide source (Figure 2). Honey contamination cannot be excluded, and possible τ-fluvalinate residues in this matrix can be calculated from the wax–honey partition coefficient (Tremolada et al. 2004). For τ-fluvalinate, these authors have proposed a value of the logarithm of the wax–honey partition coefficient (Log K_{w-h}) of 3.2. Therefore, considering the mean measured concentration in wax after 60 days (1,260 ng g^{-1}), the contamination level in honey can be estimated as 0.8 ng g^{-1}. This calculated value is below our detection limit (2.5 ng g^{-1}) and is consistent with data reported in the literature (Lodesani et al. 1992; Bogdanov et al. 1998). Taking into account this calculated contamination level, the quantity of pesticide

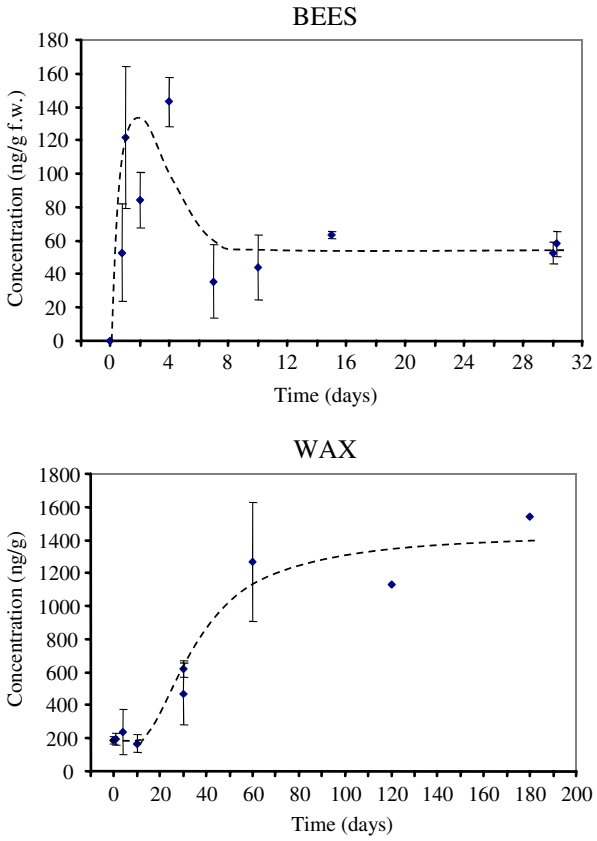

Figure 2. Mean τ-fluvalinate concentrations in bees and wax at different times after treatment. *Bars* refer to means ± standard error intervals.

residue deposited in honey is much lower than that present in bees and wax (the contribution of the honey matrix on the overall recorded amount would be 2.5% and 1% at T_4 and T_{10}, respectively). The total quantity of pesticide recovered does not substantially change when the estimated residue content of honey is added. Additional losses may occur due to bee renewal within the hive and due to the metabolic degradation of the pesticide. The average lifetime of a single bee is 60 days, of which 20 days are spent in the larval and pupal stages (Chauvin 1968). Therefore, the renewal time of adult bees is about 40 days. Considering 5,200 g of bees (40,000 bees with a weight of 130 mg per bee) with a mean contamination level of 73 ng g^{-1} from the first to the 30th day of treatment, about 0.4 mg of pesticide might be lost outside the hive through bee renewal. Other minor losses can occur through advection out of the hive via air. Considering that τ-fluvalinate has a vapour pressure of 9×10^{-11} Pa (Tomlin 1997), a saturation concentration 0.019 ng m^{-3} can be calculated for the air in the hive. Given an annual flux of air of 150 m^3, about 0.0078 ng day^{-1} would be lost from the hive through advection. This quantity represents a negligible contribution to total τ-fluvalinate losses from the hive (at 60 days after treatment, a total of 0.00047 ng would have been lost via air, corresponding to one millionth of the quantity lost due to bee renewal).

These calculations provide a precise picture of τ-fluvalinate distribution and fate in the hive. Bees take up the compound from the PVC strips by direct contact. Individual bees are initially contaminated to different degrees depending on

Table II. Mass balance of τ-fluvalinate in the hive.

Matrix	T_4				T_{10}			
	Fluva conc ng g^{-1}	Compart mass kg	Fluvalinate amount µg	Fluva %	Fluva conc ng g^{-1}	Compart mass kg	Fluvalinate amount µg	Fluva %
Bees	143	5.2	744	91	58[a]	5.2	302	16
Honey	<2.5	25	<62.5	<8	<2.5	25	<62.5	<3
Wax	48	1.5	72	9	1,077	1.5	1,615	84
Total			816	100			1,917	100

Mean concentrations (conc) at times T_4 and T_{10} were used to calculate the quantities of τ-fluvalinate and its percentages (%) in bees, honey and wax, considering the average mass of each matrix present in the hive at that time (Compart mass)

[a] At T_{10} concentrations in bees were not measured, therefore T_9 mean concentration was taken in substitution, so that an overestimation of this datum is possible

their specific activity within the hive. Later, due to the widespread contamination of the hive matrices and the food exchange and body contact among individual bees, the pesticide contamination level in bees becomes more uniform. Due to the physical and chemical properties of the compound, wax is the final sink of the pesticide residues. The compound is introduced into wax by contact with bees or by newly secreted wax that is already contaminated with the pesticide. All other organic hive matrices, including larvae, pollen and propolis, are also contaminated. These matrices receive the pesticide from the wax of the cells in which they are located and/or from the bees that produce or manipulate them. Honey does not behave differently, but it contains smaller amounts of the compound because of its hydrophilic characteristic. The pesticide does not appear to degrade readily, especially in the abiotic matrices. The biotic matrices (bees, and presumably, larvae) do not show rapid metabolic transformation. The primary means by which the pesticide is dissipated appear to be transport out of the hive through bee renewal and through the collection of products used by humans.

4. DISCUSSION

Our results are intermediate between those reported in previous studies (Bornek and Merle 1989, 1990; Tsigouri et al. 2004; Bogdanov and Kilchenmann 1995; Lodesani et al. 2003; Martel et al. 2007). The τ-fluvalinate concentrations presented here change substantially with time in both matrices (bees and wax) and between hives. The variation between times is much greater than that between hives, accounting for tenfold and twofold differences in concentration, respectively. Variation between hives depends on the sampling variability and on possible differences in the distribution of the compound within the two experimental hives. The level of variation between hives was very similar in bees and wax (mean variation coefficients of 40% in both matrices) and never exceeded 100%. τ-Fluvalinate residue concentrations reported in the literature are also variable within the same matrix. It is usual to find concentrations ranging over several orders of magnitude. Chauzat and Faucon (2007) have found a concentration range in wax from 15 to 422 ng g^{-1}, while Fries et al. (1998) have found a range from 800 to 84,000 ng g^{-1}. Several reasons can account for these differences: (a) the intrinsic variability of the sampling procedure and the non-homogeneity of the system (the differences of up to twofold in our results depend on these factors); (b) the length of time after treatment (our results show differences over time of up to tenfold); (c) the initial method of application (PVC strips avoid direct

wax contamination, while pesticide spraying into the hive deposits pesticide directly on wax) and (d) the number of treatments received by the hive (because this pesticide accumulates over time). To properly evaluate possible contamination of hive products, all of these factors must be taken into account.

The present results and those of previous studies on τ-fluvalinate residues demonstrate clearly that this compound causes diffuse contamination of hive products. In addition, the variability of the contamination levels found in each study suggests that application methods and hive-keeping practices may dramatically change the concentrations found in various hive products.

Figure 3 shows the median concentrations of τ-fluvalinate residues in the major hive matrices (bees, honey and wax) together with those of other pesticides used to protect honeybees against *Varroa*. Median concentrations of each pesticide in the various matrices are shown together on a logarithmic scale to highlight the differences between matrices and the variability among studies. The bee matrix has not been analysed often; honey and wax have been considered more frequently. A large amount of experimental data is available for τ-fluvalinate, coumaphos and bromopropylate, while malathion and amitraz are less represented. Median concentrations in wax are generally greater than 100 ng g^{-1} for τ-fluvalinate, coumaphos and bromopropylate. On the other hand, concentrations in honey are always less than 100 ng g^{-1} and often less than 10 ng g^{-1} for all compounds considered. These results are generally consistent with the physical and chemical properties and stability of the compounds (Table III). Fluvalinate, bromopropylate and coumaphos are stable lipophilic compounds that accumulate preferentially in the wax matrix. Malathion is more water-soluble and therefore, accumulates less specifically in wax relative to honey. Amitraz is a fat-soluble, unstable active ingredient whose contamination levels degrade with time (Wallner 1999). In addition to these general relationships, the most outstanding result shown in Figure 3 is the wide variability of experimental results for the same matrix and compound. Median concentrations of the same compound in the same matrix typically range over two orders of magnitude. Several factors may account for this variability, as discussed above. In particular, application modalities can be a key factor determining different contamination levels as suggested by Tsigouri et al. (2004). These factors could be included in a predictive model to assess pesticide residues in hive matrices. Such a model could include the properties of the compound, the method of application and characteristics of the hive. In this way, at a theoretical level, various application conditions could be tested in advance. Contaminants coming from outside the hive could also be considered in order to assess possible additive, synergistic or antagonistic effects in association with in-hive chemicals.

The primary goal of our research is to develop a hive model based on the experimentally derived τ-fluvalinate concentrations in bees, wax and honey. Part 2 of this report proposes a new hive model based on multi-compartmental fugacity models (Mackay 2001), which improves upon the simple model previously developed for the hive ecosystem (Tremolada et al. 2004). In particular, the static form of the previous model was unrealistic and limited prediction of the evolution over time of contamination levels. In contrast, our new model is based on a dynamic scenario.

5. CONCLUSION

Part 1 of this study demonstrates that τ-fluvalinate is a persistent contaminant of hive products (especially wax), that residue concentrations change greatly over time and that pesticide contamination differs substantially depending on application methods. A predictive model can be of great help to understand the variability in empirical results. Such a model would facilitate not only a posteriori comprehension but also predictive evaluation of pesticide contamination and of contaminants in general.

Bees are the primary vectors of unwanted pesticide distribution into other hive matrices, playing a more important role than the usual transport pathways (air and water). Bees play both a passive and an active role. Passive distribution

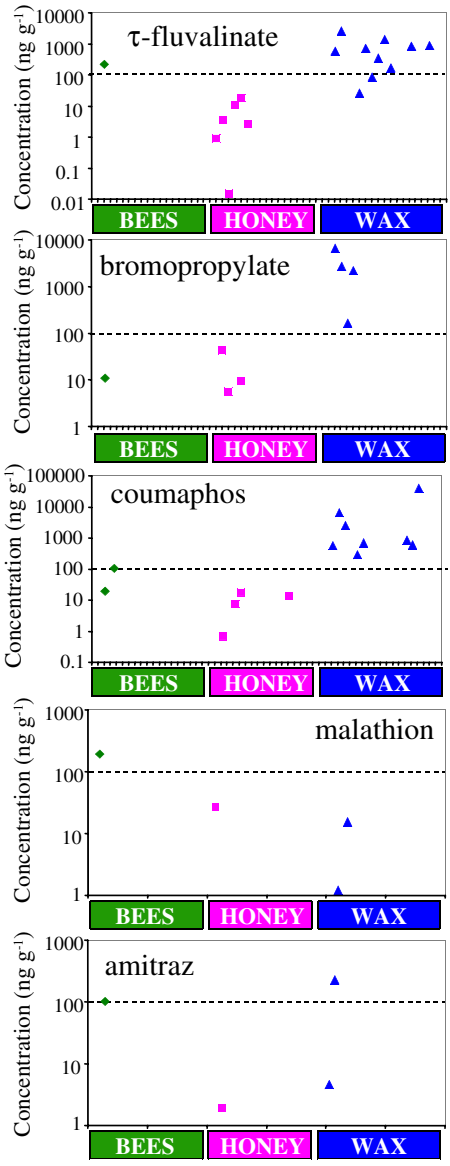

Fig. 3 Median concentrations in bees, honey and wax of the most important pesticides used to protect honeybees from *Varroa*. τ-fluvalinate data are taken from: Lodesani et al. (1992, 2003), Wallner (1997, 1999), Russo and Neri (2002), Waliszewski et al. (2003), Kamel and Al-Ghamdi (2006), Tsigouri et al. (2004), Bogdanov and Kilchenmann (1995), Bogdanov (2006), Borneck and Merle (1989, 1990), Jiménez et al. (2005), Chauzat and Faucon (2007), Friés et al. (1998), Nasr and Wallner (2003), Martel et al. (2007) and Mullin et al. (2010). Bromopropylate data are taken from: Lodesani et al. (1992), Wallner (1999), Jiménez et al. (2005) and Bogdanov (2006). Coumaphos data are taken from: Thrasyvoulou and Pappas (1988), van Buren et al. (1992), Wallner (1997, 1999), Kamel and Al-Ghamdi (2006), Jiménez et al. (2005), Chauzat and Faucon (2007), Bogdanov and Kilchenmann (1995), Friés et al. (1998), Tremolada et al. (2004), Martel et al. (2007), Bogdanov (2006), Nasr and Wallner (2003), Ghini et al. (2004), Westcott and Winston (1999) and Mullin et al. (2010). Malathion data are taken from: Thrasyvoulou and Pappas (1988), Ghini et al. (2004), Chauzat and Faucon (2007) and Mullin et al. (2010). Amitraz data are taken from: Lodesani et al. (1992), Jiménez et al. (2005) and Mullin et al. (2010)

Table III. Physical and chemical properties of the compounds considered (Tomlin 1997): molecular weight (MW), water solubility (S), vapour pressure (VP), Henry's constant (H), and logarithm of *n*-octanol/water partition coefficient (Log K_{ow})

Compound	MW g mol^{-1}	S Mol m^{-3}	VP Pa	H Pa m^3 Mol^{-1}	Log K_{ow}
τ-fluvalinate	502.9	2.05×10^{-6}	9×10^{-11} (25°C)	0.000044	4.3
coumaphos	362.8	0.0041	1.3×10^{-5} (20°C)	0.0031	4.1
bromopropylate	428.1	<0.0012	1.1×10^{-5} (20°C)	>0.0094	5.4
malathion	330.3	0.44	0.0053	0.012	2.8
amitraz	293.4	0.00034	5.1×10^{-5} (20°C)	1.06	5.5

occurs through contact of their legs and bodies with a contaminated surface (a PVC strip in the case of Apistan treatment) and subsequent diffusion of the compound within the hive (especially on the wax or wood/propolis surfaces on which they walk). Active distribution occurs when the compound is incorporated into the animal body through various ways (contact, ingestion and respiration). The compound can then be metabolised and/or transferred to bee products (honey, pollen, newly secreted wax and royal jelly). All of these products can also become contaminated through contact with wax when they are stored inside cells. Brood can be contaminated during deposition and later during feeding with residue-containing foods. All of these pathways can be modelled in a dynamic form to estimate the trends over time in the resulting pesticide concentrations in the hive products. This will be the goal of part 2.

Prédire le devenir des pesticides dans la ruche (1° partie): détermination expérimentale des résidus de τ-fluvalinate chez les abeilles, dans le miel et la cire

Fluvalinate / résidus de pesticides / contamination de la cire / contamination du miel / santé de l'abeille

Zusammenfassung – Vorhersage der Verteilung von Pestiziden im Bienenvolk (Teil I): Experimentell bestimmte tau-Fluvalinat-Rückstände in Bienen, Honig und Wachs. Mathematische Modelle werden häufig benutzt, um die Verbreitung von organischen Wirkstoffen in der Umwelt vorherzusagen. Das Bienenvolk kann dabei als Mikro-Ökosystem angesehen werden, in das chemische Kontaminationen von außen und von innen hineinkommen können, je nach Anwendung von Pestiziden zur Bekämpfung von Parasiten. Um die möglichen Kontaminationsebenen von Bienenprodukten vorhersagen zu können, haben wir ein Multi-Kompartment-Modell für das Bienenvolk entwickelt. Um die Validität eines solchen Modells zu überprüfen, sind spezifische Zeitreihenanalysen notwendig. Wir haben daher die Rückstände von tau-Fluvalinat in Bienen, Honig und Wachs in zwei mit Apistan behandelten Bienenvölkern gemessen (Figure 1). Die Rückstände in Bienen wurden über 30 Tage nach der Behandlung verfolgt, bei Honig und Wachs sogar über einen Zeitraum von 180 Tagen (Table I). Im Honig konnten keine Rückstände oberhalb der Nachweisgrenze (2.5 ng g^{-1}) gefunden werden, vermutlich wegen der geringen Wasserlöslichkeit und dem hohen *n*-Octanol-Wasser-Verteilungskoeffizienten (Log K_{ow}=4.3) des Wirkstoffes. Die Wirkstoffkonzentrationen in Bienen bewegten sich zwischen 14 und 160 ng g^{-1} f.w. (Figure 2). Vor der Behandlung waren keine Rückstände in Bienen nachweisbar. Sie stiegen dann bis zum 4. Tag an mit großen Schwankungen zwischen den einzelnen Proben, um dann vom 7. Tag an bis zum Ende der Behandlung (30 Tage) rasch zu einem fast konstanten Niveau (about 50 ng g^{-1} f.w.) abzusinken. Die Konzentrationen im Wachs lagen zwischen 98 und 1,630 ng g^{-1} (Figure 2). Im Wachs waren bereits vor der Behandlung Rückstände nachweisbar, vermutlich aufgrund früherer Behandlungen oder der Verwendung von kontaminiertem Material. Die tau-Fluvalinat-Rückstände in Wachs stiegen nach der Behandlung langsam bis zum 60. Tag an und blieben dann bis zum 180. Tag auf einem konstanten Niveau. Unsere Ergebnisse

liegen im mittleren Bereich der bisher veröffentlichten Daten anderer Untersuchungen. Das auffälligste Ergebnis unserer Literaturrecherche ist die extrem hohe Schwankungsbreite der Rückstandswerte von ein und demselben Wirkstoff in derselben Matrix (Figure 3). Diese Unterschiede können mehrere Ursachen haben: (a) Unterschiede bei der Probennahme und der Inhomogenität des gesamten Systems (bei unseren Ergebnissen waren Unterschiede bis zum Faktor 2 darauf zurückzuführen); (b) die Zeitdauer nach der Behandlung (unsere Ergebnisse zeigen zeitabhängige Unterschiede bis zum Faktor 10); (c) die Anwendungsmethode (PVC-Streifen vermeiden eine direkte Wachskontamination während das Sprühen des Wirkstoffes diesen direkt auf das Bienenwachs aufbringt); (d) die Anzahl an Behandlungen pro Bienenvolk (da dieser Wirkstoff akkumuliert). Ein Vorhersagemodell kann helfen, die Variabilität solcher empirischer Untersuchungen besser zu verstehen. Es ist daher das Hauptziel unserer Untersuchung, auf der Basis der experimentell gemessenen tau-Fluvalinat-Konzentrationen in Bienen, Honig und Wachs ein Bienenvolkmodell zu entwickeln. Im zweiten Teil dieses Berichtes wird ein solches neues Bienenvolkmodell vorgestellt.

Fluvalinat / Pestizidrückstände / Wachskontamination / Honigkontamination / Bienengesundheit.

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