Bull Environ Contam Toxicol (2009) 83:822–827
DOI 10.1007/s00128-009-9806-5

Miticide Residues in Virginia Honeys

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Received: 25 November 2008 / Accepted: 22 June 2009 / Published online: 30 June 2009

Abstract Fifty honey samples from Virginia USA were analyzed for the presence of fluvalinate and coumaphos residues. Samples were collected from hives and from bottled honey provided by beekeepers. No coumaphos or fluvalinate residues above the limit of quantification (0.05 mg/kg) were detected in any of the samples, although trace levels (<0.05 mg/kg) of coumaphos were detected in three samples from hives and trace levels of fluvalinate were found in one hive sample. No residues were detected in any of the bottled honey samples and none of the samples exceeded the US EPA tolerance levels for either miticide.

Keywords Honey · Coumaphos · Fluvalinate · Residues

The introduction of parasitic mites (*Acarapis woodi* and *Varroa destructor*) into the United States has had a profound effect on the beekeeping industry (Sammatara et al. 2000). There have been significant declines in the number of managed hives, as well as the loss of most feral colonies. In Virginia, for example, the number of beekeeper colonies has decreased by almost 50% since the introduction of the mites, and annual colony losses have averaged close to 30% over the past 10 years (*Keith Tignor, personal communication*). The high colony losses and the serious consequences of mite infestations have led to the widespread use of chemical miticides in managed hives. Beekeepers have come to rely on miticides and treat colonies on a regular basis, often without regard to actual mite infestation levels.

The excessive reliance on chemical controls has led to a number of problems, including the development of resistance in Varroa mite populations (Eischen 1995; Elzen et al. 1999). Evidence also suggests that the use of miticides can have deleterious effects on the reproductive physiology of honey bees, reducing both the ability of colonies to raise queens, as well as the ability of drones (males) to produce sperm (Fell and Tignor 2001; Haarmann et al. 2002). Sub-lethal effects from miticide use may also contribute to the increased problems of queen failure and colony loss (Burley et al. 2008).

These problems are compounded by the potential for honey and wax contamination. A continued reliance on the use of miticides increases the likelihood of honey contamination, and hence, human exposure. In Europe, the two major pesticides used for Varroa control, the organophosphate coumaphos and the pyrethroid fluvalinate have been detected in both honey and beeswax (Garcia et al. 1996; Bogdanov et al. 1998; Wallner 1999; Tsigouri et al. 2004; Bogdanov 2005). Wallner (1999), for example, reported that 80% of the wax samples from German beekeepers contained 1–10 mg/kg of the commonly used pesticides, bromine propylate and coumaphos. The continual use of compounds such as coumaphos not only increases the concentration of residues in wax, but can also lead to higher residues in honey (Wallner 1999; Kochansky et al. 2001).

The residue levels of miticides in hive products are a function of several interacting factors, including the active ingredient and its chemical properties, the quantity applied, the application technique, and the duration of treatment (Tsigouri et al. 2004; Karazafiris et al. 2008). Fortunately,

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the use of both fluvalinate and coumaphos according to label recommendations does not seem to lead to serious problems with honey contamination. Tsigouri et al. (2004) showed that maximum fluvalinate levels in honey following the use of Apistan strips were 6 μg/kg. However, they also found that the use of an agricultural formulation Mavrik (containing fluvalinate) led to significantly higher maximum concentrations (39.4 μg/kg). Similar results were found following the use of coumaphos in slow-release strip formulations (CheckMite+). Karazafiris et al. (2008) found that coumaphos could be detected in honey in the brood chamber within a day of introducing CheckMite strips, and could exceed EC maximum residue levels (0.1 mg/kg) within as little as 3 days. Residue levels as high as 0.289 mg/kg were detected in honey samples collected from frames next to the coumaphos strips. Honey samples from the honey supers, however, did not contain detectable levels of coumaphos until the last day of treatment (day 42) and residue levels were well below the established MRL at less than 0.02 mg/kg.

The potential for hive product contamination should be a concern for all beekeepers. Unfortunately few published data exist for beeswax or honey samples collected in the US. Our objective, therefore, was to analyze miticide residues in honey samples collected from beekeeper hives in Virginia and from bottled honey prepared for the retail market to determine if the residues in any honey offered for sale might exceed tolerance limits.

Materials and Methods

Honey samples were collected from hives (22) and randomly selected from bottled honey (28) submitted by beekeepers. The 22 honey samples from hives were collected directly from the comb. Twelve of these samples were collected from hives managed by two beekeepers located in Augusta and Nelson Counties in Virginia in late June 2007. Six samples were collected from three hives at each location with one sample collected from sealed honey in the brood chamber of each hive and one sample from sealed honey in a super. Both beekeepers had been treating their hives with fluvalinate for mite control. Ten honey samples were also collected from hives in the Blacksburg area. Eight were collected in the fall of 2007 from university hives that been treated with either fluvalinate or coumaphos (four each). The other two samples were collected in August 2007 from a hive owned by a local beekeeper who left Apistan (fluvalinate) strips in the hive for a year. The samples from this hive were taken from a frame in the brood nest and from a honey super. After collection, the samples were delivered to the Pesticide Residue Laboratory where they were stored in a freezer at −20°C until extraction and analysis.

The bottled honey samples were submitted by beekeepers during 2007 as part of a study on honey bacteria. After completion of the bacteria study, 25 samples of bottled honey were randomly selected from different areas of the state (Fig. 1) for miticide residue analysis. Since beekeepers who submitted the samples had no prior knowledge of the residue study, the samples were not biased by any preselection. Beekeeper names and addresses were not used and samples were only identified by area of the state.

Pesticide reference standards of coumaphos, fluvalinate, ethoprop, and aldrin were supplied by ChemService (West Chester, PA) and stock standards of 1 mg/mL were prepared in toluene. Fortification standards of coumaphos and fluvalinate were prepared at 0.5 and 10 μg/mL in ethyl

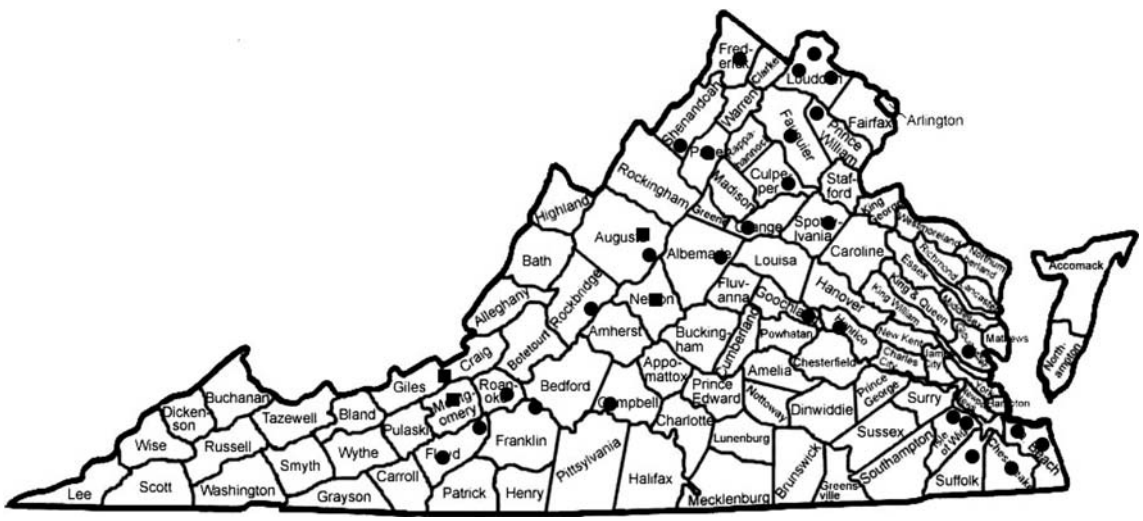

Fig. 1 Geographical distribution of honey samples collected in 2007 from hives (■) and submitted by Virginia beekeepers (●) that were used for the miticide residue analysis

acetate and these solutions were used to fortify blank honey at the 0.05, 0.1, and 0.5 mg/kg fortification levels. Matrix-matched calibration standards containing coumaphos, fluvalinate, ethoprop, and aldrin were prepared in blank honey extracts at 0.05, 0.1, 0.5, 1.0, 2.5, and 5.0 μg/mL concentrations. Quality control standards (QCSTD) were added during sample preparation to check for variability during the extraction step, and to all final extracts in autosampler vials prior to injection to evaluate variability during the gas chromatograph (GC) analytical steps. Spiked blank honey at the 0.5 mg/kg level was extracted with each analytical set and the recovery of coumaphos was 94% with a relative standard deviation (RSD) of 9% (n = 9), and the recovery of fluvalinate was 86% with an RSD of 8% (n = 9) during the course of the study.

Honey samples were extracted using a modification of the Quechers method described by Aysal et al. (2007). In brief, this modification uses ethyl acetate as the extraction solvent and GC/ECD for analysis. Prior to extraction, MgSO4 was heated at 500°C for 5 h in a muffle furnace to remove impurities, and all sorbents were pre-weighed into glass vials. Honey (5.0 g) was weighed into a 50 mL polypropylene centrifuge tube. Deionized water (10.0 mL) was added to each tube; the tube was capped, and then shaken to dissolve the honey. Ethyl acetate (15 mL) was added to the honey–water mixture in each tube. Next, 100 μL of 50 μg/mL ethoprop in ethyl acetate (QCSTD-1) was added to all tubes (except matrix blanks used to prepare calibration standards). MgSO4 (6.0 g) and NaCl (1.5 g) salts were added to the centrifuge tube and the tube was immediately capped and briefly shaken vigorously by hand to avoid clumping of salt. After salts were added to all tubes in the batch, the tubes were shaken vigorously by hand for 1 min and then centrifuged for 2 min at 5,000 rpm using a Thermo Electron IEC Model EXD centrifuge. For each tube, a 5.0 mL aliquot of the supernatant was transferred into a 5 mL polypropylene centrifuge tube. MgSO4 (750 mg) and 40 μm PSA (250 mg) were added to the tube and it was shaken by hand for 1 min and centrifuged for 2 min at 5,000 rpm. Exactly 1 mL of the supernatant was transferred to an autosampler vial prior to gas chromatographic analysis. Lastly, 200 μL of 0.3 μg/mL aldrin in ethyl acetate (QCSTD-2) was added to all final extracts in autosampler vials. Each analytical set included a reagent blank (13 mL deionized water), matrix blank (honey that had no detectable levels of pesticides—coumaphos, fluvalinate, ethoprop, and aldrin), matrix spike (0.5 μg/g), and about five Virginia honey samples.

Honey extracts were analyzed using an Agilent 6890 gas chromatograph equipped with an Agilent 7683 series autosampler, a split/splitless injector operated in the splitless mode with dual column injection (RTX-5 and RTX-35, each 30 m × 0.25 mm × 0.25 μm) to two micro-electron capture detectors (μECD). Gas flows were UHP helium carrier gas at 2.5 mL/min and UHP nitrogen makeup flow at 60 mL/min. Temperatures were injector (250°C), oven (70°C for 1 min, 20°C/min to 160°C for 1 min, 4°C/min to 275°C for 15 min), and both μECD detectors (350°C). Agilent Chemstation software version A.10.02 was used for instrument control and data acquisition, and quantification was performed using external calibration with matrix-matched calibration standards. Fig. 2 shows representative chromatograms of a reagent blank, blank honey, spiked honey at 0.5 mg/kg, and a typical honey sample.

Fig. 2 Chromatograms of **a** reagent blank; **b** blank honey; **c** honey spiked with 0.5 mg/kg of coumaphos and fluvalinate; **d** typical honey sample; all samples include quality control standards (*QCSTD*) ethoprop and aldrin

The modified Quechers method described previously was validated in-house for coumaphos and fluvalinate at 0.05, 0.1, and 0.5 mg/kg fortification levels with six replicates at each level for a total of three levels × six replicates = 18 spiked samples. For each batch of six validation samples, a reagent blank (13 mL deionized water) and a matrix blank (5.0 g) were also analyzed. The method validation characteristics of the modified Quechers method for coumaphos and fluvalinate are shown in Table 1.

The limit of detection (LOD) and limit of quantification (LOQ) were estimated according to a US Environmental Protection Agency Office of Pesticide Programs guidance document (EPA 2000). Using this calculated estimate, the LOD = SD × *t*-statistic, where SD is the standard deviation of n fortification samples spiked at the lowest limit of method validation (LLMV), and *t*-statistic refers to the one-tailed statistic available in tables. The LOQ is calculated as: LOD × 3. For coumaphos and fluvalinate, the LOD was 0.02 mg/kg and the LOQ was 0.05 mg/kg.

Results and Discussion

No coumaphos or fluvalinate residues above the limit of quantification (LOQ) of 0.05 mg/kg were detected in any of the 50 honey samples. However, trace levels (<0.05 mg/kg) of coumaphos were detected in two samples collected directly from the comb (one from a brood frame and one from a super frame) in hives managed by each of the beekeepers located in two Virginia counties, and in one of the honey samples from the treated university hives (brood frame). The honey sample from the university hive also contained trace levels of fluvalinate. No coumphos and fluvalinate residues were detected in any of the Virginia honey samples randomly selected from bottled honey submitted by beekeepers for bacterial analysis. These samples represent diverse geographical regions within

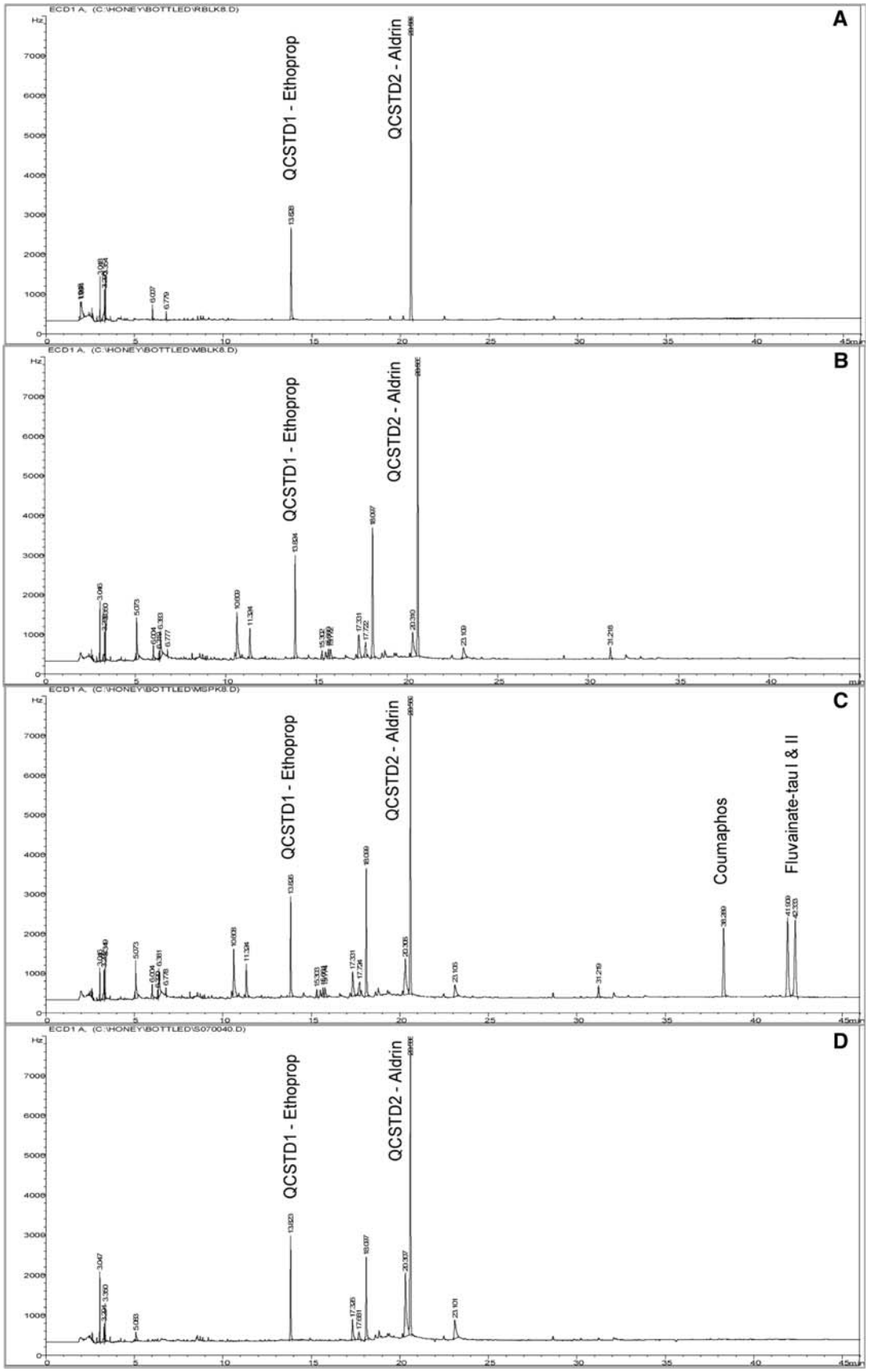
A
QCSTD1 - Ethoprop
QCSTD2 - Aldrin
B
QCSTD1 - Ethoprop
QCSTD2 - Aldrin
C
QCSTD1 - Ethoprop
QCSTD2 - Aldrin
Coumaphos
Fluvainate-tau I & II
D
QCSTD1 - Ethoprop
QCSTD2 - Aldrin

Table 1 Method validation characteristics of the modified Quechers method for coumaphos and fluvalinate in honey (n = 48)

Spiking level (mg/kg)	Accuracy		Precision	
	Average recovery (%)	Codex acceptable range[a]	Repeatability of recoveries (% RSD)	Codex acceptable range
0.05	71	70–120	7	20
0.1	79	70–120	6	20
0.5	116	70–120	8	15

[a] Codex acceptable range (Codex 1993)

Virginia and may reflect beekeeper practices in which miticides are applied according to the label instructions.

To our knowledge, no other published reports of pesticide residues in Virginia honey are available for comparison, and few published data exist for honey samples collected in the US. Our results showing the presence of low levels of coumaphos and fluvalinate in honey are similar to those reported from countries in Europe. Garcia et al. (1996), for example, reported low levels of coumaphos and fluvalinate in 44% of 221 honey samples from 1988–1991 in Spain. In one case, fluvalinate was detected in honey from a hive with a long-term exposure to Apistan strips. Two of the Virginia honey samples were collected in August 2007 from a hive owned by a local beekeeper that left Apistan strips in the hive for a year. No coumaphos or fluvalinate were detected in either of these honey samples. According to Wallner (1999) coumaphos was the most frequently detected miticide in honey at low levels between 0.002 and 0.015 mg/kg from 1995 to 1997 in Germany. Fluvalinate was less frequently detected in the same samples, but also at low levels between 0.002 and 0.007 mg/kg. Research studies that model the transfer of coumaphos from contaminated beeswax to honey (Wallner 1995; Kochansky et al. 2001; Karazafiris et al. 2008) show that coumaphos has the greatest potential to contaminate honey compared to the other pesticides studied because it is not highly lipophilic and tends to readily diffuse into honey. By comparison, fluvalinate is highly lipophilic and accumulates in wax although it also tends to diffuse into honey (Adamczyk et al. 2007). Within the scope of this study, a similar pattern was observed in which coumaphos was detected in more Virginia honey samples than fluvalinate.

According to the Federal Register on May 23, 2007 (Volume 72, Number 99), no USDA Pesticide Data Program data are available for coumaphos in honey. However, monitoring for coumaphos in honey is conducted under the Food and Drug Administration CFSAN (Center for Food Safety and Applied Nutrition) Surveillance Monitoring Program. From 1999–2006, the FDA CFSAN detected coumaphos in US honey only once in 2003 and the amount detected was below the level of quantification. During the same time period, no fluvalinate was detected in any US honey samples tested by the FDA CFSAN. Continued monitoring of pesticides in commercial honey is warranted as beekeepers implement new practices to control varroa mites and other hive pests.

The US EPA tolerance level (maximum residue level) for coumaphos is 0.15 ppm, so the trace levels (<0.05 ppm) in three Virginia honey samples are within allowable concentrations. In 2007 when this residue study began, the EPA tolerance level for fluvalinate in honey was 0.05 ppm. Only one of the 50 honey samples in this study had trace levels of fluvalinate below the LOQ of 0.05 mg/kg. Recently, the EPA tolerance level for fluvalinate was lowered to 0.02 mg/kg which indicates ongoing concern about the potential for hive product contamination. The adoption of more sustainable integrated pest management practices, and the use of fluvalinate and coumaphos according to label recommendations, will help to minimize possible honey contamination by miticides in Virginia and other honeys.

Acknowledgments The authors thank Derek Monthei for laboratory assistance and Nancy Adamson for assistance in collecting hive samples. This work was supported by a grant from the US EPA on Reduced Pesticide Use in Bee Hives (X8-973376).

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