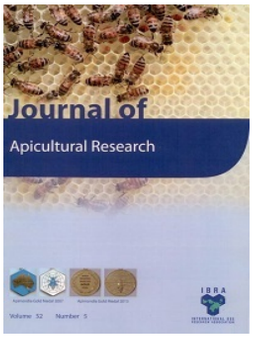


Journal of Apicultural Research

ISSN: 0021-8839 (Print) 2078-6913 (Online) Journal homepage: https://www.tandfonline.com/loi/tjar20

# Overwintered brood comb honey: colony exposure to pesticide residues

Nancy Ostiguy & Brian Eitzer

**To cite this article:** Nancy Ostiguy & Brian Eitzer (2014) Overwintered brood comb honey: colony exposure to pesticide residues, Journal of Apicultural Research, 53:3, 413-421, DOI: 10.3896/IBRA.1.53.3.10

**To link to this article:** https://doi.org/10.3896/IBRA.1.53.3.10

Published online: 10 Jul 2015.

Submit your article to this journal

Article views: 444

View related articles

View Crossmark data

Citing articles: 11 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=tjar20

*Journal of Apicultural Research* 53(3): 413-421 (2014)
DOI 10.3896/IBRA.1.53.3.10
© IBRA 2014

ORIGINAL RESEARCH ARTICLE

# Overwintered brood comb honey: colony exposure to pesticide residues

**Nancy Ostiguy[1*] and Brian Eitzer[2]**

[1]Department of Entomology, 501 ASI Bldg., Penn State University, University Park PA 16802, USA.
[2]Department of Analytical Chemistry, The Connecticut Experiment Station, 123 Huntington Street, New Haven, CT 06504, USA.

Received 23 November 2010, accepted subject to revision 26 July 2012, accepted for publication 23 August 2013.

*Corresponding author: Email: nxo3@psu.edu

## Summary

To address beekeeper concerns about pesticide residues in overwintered honey, paired samples were obtained from the extracted supers and the brood chamber of the same colony. Only eight residues were detected: coumaphos, fluvalinate, boscalid, dimethoate, atrazine, bentazon, dichlorobenzene and thymol. Honey from extracted supers was significantly less likely to contain pesticide residues than honey from brood comb. Fluvalinate was detected only in overwintered brood comb honey, and coumaphos was found at significantly higher levels in the overwintered samples from the brood comb-honey super pairs. Pesticide residues in honey, while low in comparison to other substrates in the hive, contribute to the overall pesticide exposure of honey bees, with overwintered brood comb honey contributing more than honey stored in other locations in the hive.

# Miel para la hibernada en los panales de cría: exposición de la colonia a los residuos de plaguicidas

### Resumen

Para abordar las preocupaciones de los apicultores acerca de los residuos de los plaguicidas en la miel de hibernada, se obtuvieron muestras tanto de las alzas extraídas como de las cámaras de cría de la misma colmena. Solo se encontraron 8 residuos: cumafós, fluvalinato, boscalid, dimetoato, atrazina, bentazona, diclorobenceno y timol. La miel extraída de las alzas fue significativamente menos propensa a contener residuos de plaguicidas que la extraída de los panales de cría. El fluvinato se detectó solo en la miel de hibernada de los cuadros de cría y el cumafós apareció en niveles significativamente mayores en las alzas extraídas. Los residuos de plaguicidas en la miel, aunque fueron menores en comparación con los que aparecen en otros sustratos de la colmena, contribuyen al total de exposición de las abejas a los plaguidicidas. La miel para la hibernada de los panales de cría tiene mayor cantidad de residuos que la miel almacenada en otras localizaciones de la colmena.

**Keywords:** pesticides, residues, honey, coumaphos, fluvalinate, *para*-dichlrobenzene, thymol

## Introduction

Losses of honey bee (*Apis mellifera* L.) colonies in the USA have been significant since 2006, with average winter losses reaching 30% per year (vanEngelsdorp *et al.*, 2010; 2011; 2012; Spleen *et al.*, 2013; Steinhauer *et al.*, 2014). The suspected cause of these losses is varied and range from starvation and unusual weather to pesticides and Colony Collapse Disorder (CCD). Most researchers believe the cause of elevated winter losses and CCD is multi-factorial, focusing on pathogens, stress and pesticide exposure (Cox-Foster *et al.*, 2007; vanEngelsdorp *et al.*, 2009; Neumann and Carreck, 2010).

Whilst several new pathogens have been proposed as potential factors in colony losses, including Israeli acute paralysis virus, deformed wing virus, *Nosema ceranae,* and an iridovirus (Chen and Huang, 2010; Bromenshenk, 2010), the role of pesticide exposure has been of greater concern to beekeepers (Conrad, 2009a; Conrad, 2009b). Interestingly, Alaux, *et al.* (2010) report that interactions are possible between pesticides and pathogens; imidacloprid and *Nosema* spp. interact to weaken bees. An extensive survey of bees and hive products by Mullin *et al.* (2010) found 121 different pesticides and metabolites in samples obtained from three East Coast migratory beekeeping operations, from beekeepers experiencing symptoms of CCD, or colonies

exposed to pesticides during apple pollination. Fluvalinate and coumaphos, both found in 98% of the wax samples, are used by beekeepers to control varroa mites. The range of fluvalinate and coumaphos residues ranged from 2.0 µg/Kg to 204 mg/Kg and 1.0 µg/Kg to 919 mg/Kg, respectively.

In various studies over the last two decades, residues of acaricides, including Apistan®, Mavrik® and Klartan® (fluvalinate), CheckMite®, Perizin®, and Asuntol®50 (coumaphos), Apivar® (amitraz), Bayvarol® (flumethrin), Folbex® (bromopropylate), Apiguard® (thymol), and Apilife Var® (thymol and other essential oils), and other materials used in beekeeping, e.g. *para*-dichlorobenzene, have been reported in hive products such as wax, honey and propolis (Lodesani *et al.*, 1992; Bogdanov *et al.*, 1998a; 1998b; Wallner, 1999; Bogdanov *et al.*, 2006; Tremolada *et al.*, 2004; Martell *et al.*, 2007).

Residues in adults and brood have also been reported. In a study of the distribution of pesticides in bees and hive products, Smodis Skerl *et al.* (2010) reported fluvalinate in bee heads and larvae after a single colony exposure. Mullin *et al.* (2010) analysed 140 bees and found that 83.6% of the bees contained fluvalinate while 60% contained coumaphos. The maximum amount of fluvalinate or coumaphos residues detected was 5860 µg/Kg and 762 µg/Kg, respectively, and substantially below the honey bee $LD_{50}$ for these compounds (15860 µg/Kg and 46300 µg/Kg, respectively). Of greater concern than the individual $LD_{50}$ for each compound is the potential for interactions, since the majority of bees contained both chemicals. Johnson *et al.* (2009) reported higher toxicity in adults to fluvalinate after pre-exposure to coumaphos.

To address beekeeper concerns about the potential impact of pesticides on bee health, samples of honey were obtained from the extracted supers and the brood chamber of the same colony. Honey from extracted supers was sampled in the fall, whilst brood chamber honey was sampled in the spring. The sample of honey from supers provides information on pesticide residues primarily obtained from flowers by foraging bees, whilst the brood comb honey reflects pesticide residue levels fed to brood and consumed by adult overwintering bees.

# Material and method

In 2007, letters were sent to all State beekeeping associations in the USA requesting samples of honey. Each beekeeper was asked to provide 90-150 ml of extracted and brood comb honey creating an extracted honey (from supers) - brood comb honey pair. Honey from the extracted supers was sampled in Fall 2007. To reflect the honey the bees would consume during winter, brood comb honey was removed in early Spring 2008 from the same colonies from which extracted honey was obtained in the fall. All beekeepers had small (less than 20 hives) operations. Beekeepers from Colorado, Connecticut, Illinois, Louisiana, and Wisconsin sent samples of extracted and brood comb honey to The Pennsylvania State University in Spring 2008. Only extracted honey was obtained from Louisiana. The Louisiana samples included honey extracted from supers in 1995, 1996, 1999, 2000, 2001, and 2005-2008. A sample of extracted honey from Pennsylvania was used a negative control since it was known to have been extracted from hives never treated with acaricides, dichlorobenzene or any other compound. All colonies were located in suburban or rural areas with a mix of wild and agricultural lands.

Honey samples were split into duplicates at the Pennsylvania State University and one duplicate was sent to The Connecticut Agricultural Experiment Station (CAES) for pesticide analysis. All 38 samples were extracted using a modified version of the QuECHERs procedures originally developed by Anastassiades *et al.* (2003). Since then many versions of these procedures have been tested and put into widespread use with exact modifications based on specific analytic lists and available instrumentation. The procedures in use at the CAES have been reported in their modified form by Krol *et al.* (2010). In brief, a 3 g sample of honey was spiked with an isotopically labelled standard; 12 ml of water was added and then the sample was extracted with 15 ml of acetonitrile in the presence of 6 g $MgSO_4$ and 1.5 g of sodium acetate. The sample was then mixed and centrifuged. Ten ml of the supernatant was combined with 0.5 g PSA, 0.5 g C-18 silica, 1.5 g $MgSO_4$ and 2 ml toluene. Again the sample was mixed and centrifuged; 6 ml of the supernatant was concentrated to 1 ml for LC/MS/MS analysis. These analyses were conducted using an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 column (2.1 x 150 mm, 5 µm particle size) interfaced to a Thermo LTQ ion trap mass spectrometer. The LC solvents were water and methanol each containing 0.1% formic acid. The gradient program ran from 12.5% methanol to 100% methanol over a 22-minute period. The LC/MS/MS targets 140 different pesticide residues. For each pesticide, scan specific MS/MS transitions are monitored to have unambiguous identification of that pesticide. To insure good quantitation, the 140 different scan events were separated into three instrument methods (3 separate injections of the extract). Although detection limits vary with the individual pesticide, for most of these compounds the limits are less than 5 mg/Kg; the specific limits for the compounds found are reported in Table 1.

Thymol and dichlorobenzene were two compounds of interest that could not be analysed by the QuECHERs procedures. We attempted to analyse these compounds by a solid phase microextraction technique (SPME). A 1 g sample was combined with 1 ml of water in a vial and the headspace gas was sampled for 30 min using a carboxen-PDMS fibre assembly. The SPME needle was then transferred to the injection port of an Agilent 5973 GC/MSD system for analysis on a 30 m x 0.25 mm DB-5 column. The GC was temperature program was as follows 40ºC for 4 min, 3ºC/min to 55ºC, 5ºC/min to 120ºC, 10ºC/min to 180ºC, 20ºC/min to 260ºC and hold for 4 min. However, these compounds gave inconsistent results using this technique and therefore we only used the data qualitatively to get an indication of the presence or absence of these compounds.

***Table 1.*** Quantified pesticide residues in extracted (Fall 2007) and overwintered brood comb honey samples (Spring 08). Samples from beekeepers residing in Colorado, Connecticut, Illinois and Wisconsin were analysed using QuECHERs procedures. The Pennsylvania samples represent a negative control. Compounds listed as not detected (ND) were below the limits of detection (0.4 µg/kg for coumaphos, 1 µg/kg for the other five pesticides).

| Sample Pairs | Honey Source | | Coumaphos µg/kg | Fluvalinate µg/kg | Boscalid µg/kg | Dimethoate µg/kg | Atrazine µg/kg | Bentazon µg/kg |
|---|---|---|---|---|---|---|---|---|
| Wisconsin A | Extracted | | ND | ND | ND | **11** | ND | ND |
| | Brood area | | ND | **6.8** | ND | **23** | **1.6** | ND |
| Wisconsin B | Extracted | | **16.0** | ND | ND | ND | ND | ND |
| | Brood area | | ND | ND | ND | ND | ND | ND |
| Colorado A | Extracted | | **2.8** | ND | ND | ND | ND | ND |
| | Brood area | | **3.0** | ND | ND | ND | ND | **25** |
| Colorado B | Extracted | | ND | ND | ND | ND | ND | ND |
| | Brood area | | **37.0** | **23** | ND | ND | ND | ND |
| Illinois A | Extracted | | ND | ND | ND | ND | ND | ND |
| | Brood area | | ND | ND | ND | ND | ND | ND |
| Illinois B | Extracted | | **6.1** | ND | ND | ND | ND | ND |
| | Brood area | | **210.0** | ND | ND | ND | ND | ND |
| Connecticut | Extracted | | **1.1** | ND | ND | ND | ND | ND |
| | Brood area | 1 | **0.5** | ND | ND | ND | ND | ND |
| | | 2 | **1.2** | ND | ND | ND | ND | ND |
| | | 3 | **280.0** | **8** | ND | ND | ND | ND |
| | | 4 | **23.0** | **3.2** | ND | ND | ND | ND |
| | | 5 | **49.0** | **26** | ND | ND | ND | ND |
| | | 6 | **164.0** | **11** | ND | ND | ND | ND |
| | | 7 | **45.0** | ND | ND | ND | ND | ND |
| | | 8 | **3.0** | ND | ND | ND | ND | ND |
| | | 9 | **280.0** | ND | ND | ND | ND | ND |
| | | 10 | **62.0** | **12** | ND | ND | ND | ND |
| | | 11 | **13.0** | ND | **2.3** | ND | ND | ND |
| Pennsylvania | Extracted | | **0.7** | ND | ND | ND | ND | ND |

# Results

Louisiana and Connecticut contributed the majority of the samples tested: 38% and 32%, respectively (Table 1). Wisconsin, Colorado and Illinois each contributed 10.5% of the samples. All the historical samples were from extracted honey supers in Louisiana, except for the negative control from Pennsylvania.

Six compounds were detected and quantified using QuECHERS (Table 1). Two compounds, thymol and dichlorobenzene, were detected by SPME (Tables 2 & 3). Of the quantifiable pesticides detected, the greatest number of detections per sample was 3 (fluvalinate, dimethoate and atrazine), which was observed in the honey from the overwintered brood comb (Table 1). Only one extracted honey - brood comb honey pair contained no quantifiable pesticides. A single pesticide was quantified in 47.4% of the samples while 21% of samples contained two pesticides. Honey from supers was significantly less likely to contain residues than honey from brood comb ($t = 2.94$, $df = 21.725$, $p = 0.010$).

Coumaphos, the active ingredient in CheckMite®, was detected in 63% of the samples; 82% of the brood comb honey and 47% of the honey from supers contained coumaphos residues. When all samples of extracted honey from supers (1.47 ± 3.61) were compared to all samples of brood comb honey (67.1 ± 99.62), coumaphos concentrations were significantly lower in the honey extracted from supers (Satterthwaite $t = 2.71$, $df = 16$, $p = 0.015$). If coumaphos was found in an extracted honey (3.70 ± 5.86) - brood comb honey (67.1 ± 99.6) pair, coumaphos was significantly more likely to be found in the brood comb honey (paired $t = 2.65$, $df = 17$, $p = 0.017$). None of the honey from Louisiana prior to spring 2005 contained coumaphos. However, coumaphos was detected at very low levels (0.4-1.0 µg/Kg) in extracted honey from Louisiana beginning in 2005 (Table 3); CheckMite® has been allowed under a Section 18 Emergency Use approval since 1999 but no colonies in Louisiana were ever treated with CheckMite®.

Fluvalinate, the active ingredient in Apistan®, was found in 18.4% of the samples and only in brood comb honey. It was not found in any of the historical extracted samples from Louisiana or the negative control from Pennsylvania.

Atrazine, boscalid and bentazon were found once in each of three separate overwintered honey samples. Dimethoate was found in one historical extracted honey sample (Table 2: Louisiana 2001-B) and in a single extracted honey-brood comb honey pair with higher levels observed in the brood comb honey (Table 2: Wisconsin-A).

***Table 2.*** Extracted honey from Louisiana. No acaricides were used in the colonies/hives from which the honey was extracted. Coumaphos and dimethoate were detected using the QuECHERs procedures (µg/Kg) and Solid Phase Extraction was used to detect dichlorobenzene (DCB) (P = present; ND = nondectectable).

| YEAR | Fall/ Spring | Coumaphos | Dimethoate | DCB-by PME |
|---|---|---|---|---|
| 1995 | Fall | ND | ND | ND |
| 1996 | Spring | ND | ND | ND |
| 1999 - A | Spring | ND | ND | ND |
| 1999 - B | Spring | ND | ND | P |
| 2000 - A | Spring | ND | ND | ND |
| 2000 - B | Spring | ND | ND | P |
| 2001 - A | Spring | ND | ND | P |
| 2001 - B | Spring | ND | 1.4 µg/kg | ND |
| 2005 - A | Spring | 1.0 µg/kg | ND | P |
| 2005 - B | Spring | 1.0 µg/kg | ND | ND |
| 2006 | Spring | 0.7 µg/kg | ND | ND |
| 2007 | Spring | 1.0 µg/kg | ND | ND |
| 2008 | Spring | 0.4 µg/kg | ND | ND |

***Table 3.*** Solid Phase Micro Extraction pesticide residues. Beekeepers from Colorado, Connecticut, Illinois and Wisconsin provide honey from extracted supers (Fall 2007) and overwintered brood comb (Spring 08). The Pennsylvania sample was a negative control as no chemicals were used in the colonies/hives from which honey was extracted. (P = present; ND = nondetectable).

| Sample Pairs | Honey Source | | Dichlorobenzene | Thymol |
|---|---|---|---|---|
| Wisconsin A | Extracted | | ND | ND |
| | Brood area | | ND | ND |
| Wisconsin B | Extracted | | **P** | ND |
| | Brood area | | **P** | ND |
| Colorado A | Extracted | | **P** | ND |
| | Brood area | | **P** | ND |
| Colorado B | Extracted | | ND | ND |
| | Brood area | | **P** | ND |
| Illinois A | Extracted | | **P** | ND |
| | Brood area | | **P** | ND |
| Illinois B | Extracted | | ND | ND |
| | Brood area | | ND | **P** |
| Connecticut | Extracted | | **P** | ND |
| | Brood area | 1 | **P** | **P** |
| | | 2 | **P** | ND |
| | | 3 | ND | ND |
| | | 4 | **P** | ND |
| | | 5 | **P** | ND |
| | | 6 | ND | ND |
| | | 7 | ND | ND |
| | | 8 | **P** | ND |
| | | 9 | **P** | **P** |
| | | 10 | ND | ND |
| | | 11 | ND | ND |
| Pennsylvania | Extracted | | ND | ND |

Although the SPME data were inconsistent, there is some evidence of the presence of dichlorobenzene in the samples. The residues (if actually present) were extremely low (likely less than µg/Kg). A couple of samples also appeared to contain thymol residues but again the quality of the data lead us only to tentatively report the presence or absence of this compound. The method for these compounds needs to be refined. Tables 2 & 3 therefore present only the potential presence of these compounds. Dichlorobenzene was potentially present in 30% of the historical extracted honey samples from Louisiana (Table 2) and in 56% of the extracted-comb honey pairs (Table 3). The potentially positive samples included honey from brood comb and honey from honey supers. Thymol was not observed in any of the Louisiana historical samples (Table 2) and it was potentially detected in 8% of the samples from other locations and only in honey from brood comb (Table 3).

# Discussion

We found significantly fewer pesticide residues than reported by Mullin *et al.* (2010), Choudhary and Sharma (2008) and Blasco *et al.* (2003). Four residues from compounds used by beekeepers, including fluvalinate, coumaphos, thymol and dichlorobenzene, were detected along with residues of four pesticides not typically used in apiculture (Table 2). This difference is not unexpected since Mullin *et al.* (2010) reported on residues found in wax, pollen and bees, all of which have higher lipid content than honey and many of the pesticides residues they reported are lipophilic. Additionally, our samples were from small beekeeping operations while most of the samples tested by Mullin *et al.* (2010) were from large or migratory operations. Differences in the foraging landscape may explain the greater number of residues found be Choudhary and Sharma, (2008) and Blasco *et al.* (2003) but this information is unknown. Chauzat *et al.* (2010) in a study of colony health in France reported similar levels of coumaphos and fluvalinate residues in honey similar to those reported in this study but no non-acaricide residues were detected. Thus pesticide residues in honey can vary from none to acaricide only to acaricide and agricultural pesticide.

A number of recent studies reported a greater number of agricultural pesticide residues at sub-acute concentrations than we observed. Honey from Himachal Pradesh India contained residues of a variety of non-acaricides including DDE, DDD, DDT, HCH, dimethoate, malathion, quinalphos, cyhalothrin, cypermethrin, deltamethrin, dicofol, endosulfan, and fenvalerate (Choudhary and Sharma, 2008). Samples of honey from Portugal and Spain were reported to have residues of DDT, hexachlorohexane (HCH), hexachlorbenze, heptenophos, methidathion, methyl parathion, carbofuran, primicarb, methiocarb and carbaryl (Blasco *et al.*, 2003).

Agricultural chemicals, in addition to acaricides, are found at low concentrations in honey. Generally, honeys from *Apis mellifera* colonies contained more residues than honey from *A. ceranae* colonies; the

authors attribute this result to a greater percentage of forage for *A. ceranae* being from non-agricultural crops (Choudhary and Sharma, 2008). Similar results were reported by Sarfraz Khan *et al.* (2004) for *A. mellifera* versus *A. dorsata* and *A. florea*. A greater number and higher concentration of residues were found in *A. mellifera*. The residues detected included aldrin acephate, carbaryl, chlorpyriphos, DDT, endosulfan, dimethoate, endosulfan, HCH, heptachlor, malathion, methyl-parathion and quinalphos.

The evidence supporting a relationship between sub-acute exposure to pesticide residues in honey and current colony health problems are mixed. While the most recent problems with colony loss have been occurring for seven years (Cox-Foster *et al.*, 2007), the types of residues present in honey have been consistent for more than a decade. Al-Rifai and Akeel (1997) reported, 2, 4-DDD, 2,4-DDT, 44-DDT aldrin, bromophos methyl, dichlorvos, dieldrin, fenitrothion, fluvalinate, hexachlorocyclohexane (alpha and beta), heptachlor, heptachlorepoxide, lindane and mervinphos in domestically (Jordan) produced honey and aldrin, DDT, dieldrin heptachlor, heptachlorepoxide, HCH, fluvalinate, and lindane were detected in imported honey samples. Fernández-Muiño *et al.* (1995), in a study of non-acaricide pesticides residues in honey from seven European countries (Bulgaria, Denmark, Finland, Germany, Italy, Poland, and Spain), reported detection of aldrin, DDE, DDT, dialiphos, dichlorvos, dieldrin, endosulfan, heptachlor lindane, methoxychlor, and vinclozolin. A limited pesticide analysis of 21 samples honey in Spain reported no amitraz, bromopropylate, fluvalinate, or tetradifon but coumaphos, ethion, and thiaxolin were detected (Avila *et al.,* 1990).

Bogdanov (2006) and Wallner (1999) report similar µg/Kg levels of coumaphos and fluvalinate in honey to those reported here while Chauzat *et al.* (2010) detected coumaphos in only one sample (0.2 mg/kg) and the single sample of fluvalinate residue was below the limit of quantification. Wallner (1999) reported that 28% of extracted honey contained coumaphos residues between 2 and 15 µg/Kg. A higher percentage of our extracted honey samples (47%) were positive for coumaphos though the range (0.4-16.0 µg/Kg) of residues was quite similar (Tables 1 & 2). The more frequent detection may be a result of greater use of coumaphos by beekeepers or greater contamination of honey bee forage. Interestingly, coumaphos was found in honey from hives where coumaphos had never been used by the beekeeper (Table 2). While the range of residues in this subset of samples was low (0.4-1.0 µg/Kg), this suggests that some portion of the coumaphos residue in hives may result from contamination of nectar collected by honey bees or the use contaminated foundation wax in the supers (see below).

Coumaphos and fluvalinate residues observed in the overwintered brood comb honey were higher than in extracted honey. This may be due to the direct contamination of honey from use of coumaphos and/or fluvalinate in the brood chamber for mite control in Fall 2007 or from indirect contamination of honey from wax contaminated with these acaricides as a result of their use in the brood chamber in previous years (Wallner, 1995). Coumaphos and fluvalinate are fat-soluble compounds and will accumulate in bees wax. In fact, these compounds have even been observed in new foundation prepared from old wax (unpublished data; Mullin *et al.* 2010). If the concentration of either chemical in the wax is high enough, the migration from bees wax to honey is possible. Kochansky *et al.* (2001) discusses the migration of coumaphos from wax to syrup. Using their data on the relationship between coumaphos in syrup and wax at equilibrium, the estimated residue of coumaphos in the wax from the colonies in our study was 10 mg/Kg or less in extracted supers and between 10 and 1000 mg/Kg in the brood comb. *Tremolada et al.* (2004) stated that 0.23% of coumaphos and 0.2% of fluvalinate in wax can move into honey. Extrapolating this information to our samples, coumaphos in wax is predicted to be as high as 8.5 mg/Kg in the wax from the honey supers and 122 mg/Kg in wax from the brood comb. Fluvalinate residues in wax would be predicted to be as high as 13 mg/Kg in brood comb. These concentrations are well within the range of the data reported by Mullin *et al.*, 2010.

Only a few non-beekeeping related pesticides were detected in our samples (Table 1) and their presence can most likely be explained by residues in the pollen and the nectar collected by honey bees while foraging. Additionally, the number of detections per pesticide was low. Dimethoate was the only pesticide detected more than once. The toxicity to honey bees of these non-beekeeping related pesticides varies from high to low. The $LD_{50}$ of dimethoate is 1.2-1.5 ·g/bee (Aupinel *et al.*, 2007). The 48-hour oral $LD_{50}$ of dimethoate is ~1.9 µg for a 4-day larva but at day 7, the larval oral $LD_{50}$ is 2.5 mg/Kg. If the average weight of a honey bee is assumed to be ~0.218 g, honey with ~5.5 mg/Kg dimethoate would be toxic to an adult bee. All dimethoate positive samples (2) were an order of magnitude less than the concentration needed to produce bee mortality (Table 1). A single sample of brood comb honey contained 2.3 µg/Kg boscalid. With an oral and contact $LD_{50}$ for boscalid greater than 100 µg/bee (Australian Pesticide and Veterinary Authority, 2004), toxicity to adult or immature honey bees from boscalid is unlikely. The other compounds detected, atrazine and bentazon, are non-toxic to adult honey bees (Meister, 1992; Hallenbeck and Cunningham Burns, 1985).

Fluvalinate and coumaphos, two of the most commonly used chemical in beekeeping, are toxic to honey bees if the concentration is sufficiently high. The $LD_{50}$ of coumaphos and fluvalinate to an average honey bee is ~14.4 µg and ~9.5 mg, respectively (Harding, 1974; Atkins, 1992). Honey with ~660 mg/kg coumaphos or ~430 mg/kg fluvalinate would be toxic to bees. The impact of fluvalinate or coumaphos on the immature stages of honey bees is unknown. Recent work by Johnson *et al.* (2009) indicate a synergistic response between fluvalinate and coumaphos. Three-day-old larvae exposed to coumaphos prior to fluvalinate exposure resulted a large increase in fluvalinate toxicity while exposure to fluvalinate prior to exposure to coumaphos

resulted in a moderate increase in coumaphos toxicity. Thus it may be possible that simultaneous exposure to the compounds from both honey and wax may result in a toxic exposure to immature bees.

Numerous studies have shown that residues in honey are not likely to be found at levels that would be acutely toxic to honey bees (Chauzat *et al.*, 2010; Blasco *et al.*, 2003; Choudhary and Sharma, 2008; Al-Rifai and Akeel, 1997; Fernández-Muiño *et al.*, 1995; Avila *et al.*, 1990). Therefore, it is important to evaluate the potential for sub-acute to chronic effects that may occur in honey bee colonies. Chronic/sub-acute exposure of honey bees to imidacloprid, its metabolites (5-OH-imidacloprid) and other neonicotinoids have been reported to cause cytotoxicity in the malpighian tubules, neuronal inactivation, decreased size hypopharyngeal gland acini in 1 week old bees, and reduced olfactory learning performance, with summer bees showing a greater decrease than surviving winter bees (De Almeida Rossi *et al.*, 2013; Hatjina *et al.*, 2013; Palmer *et al.*, 2013; Williamson and Wright, 2013; Heylen *et al.*, 2011; Colin *et al.*, 2004; Decourtye *et al.*, 2003). Aliouane *et al.* (2009) exposed honey bees to fipronil, acetamiprid, or thiamethoxam for 11 days at sublethal doses ranging from 0.2-20% of the $LD_{50}$. Fipronil, acetamiprid and thiamethoxam reduced motor, sensory and cognitive function. Decourtye *et al.* (2005) demonstrated reduced learning by honey bees when exposed to sublethal doses of fipronil, deltamethrin, endosulfan and prochloraz. The quantity feed daily to each bee was the oral 48-hour $LD_{50}$ of the compound divided by 20. No impact on behaviour was observed following treatment with λ-cyalothrin, cypermethrin, τ-fluvalinate, triazamate, or dimethoate. Fipronil has been shown in older Africanized honey bees to impact mushroom bodies thereby affecting metabolism by increased mitochondrial respiration (Roat *et al.*, 2013). After exposure was discontinued, neural activity did not recover. Coumaphos, an organophosphate pesticide used within honey bee colonies to kill varroa mites and a contaminant of hives resulting from foraging activities (see Table 2), adversely impacts learning, causes neuronal inactivation, and reduces trophallaxis (Williamson and Wright, 2013; Palmer *et al.*, 2012: Bevk *et al.*, 2012). A fungicide (captan), and two insecticides (indoxacarb and fenoxycarb) decreased the size hypopharyngeal gland acini (captan and indoxacarb) and hypopharyngeal disintegration (fenoxycarb only); hypopharyngeal glands of newly emerged honey bees treated with sublethal doses fenoxycarb were similar to bees at the onset of foraging activity (Heylan *et al.*, 2010).

The EPA sets tolerance levels for pesticides that may be present in food to protect human health. If a pesticide is used according to the label, tolerances should not be exceeded. The tolerance for coumaphos is 150 µg/kg in honey and 45 mg/Kg in honey comb (CFR Title 40 part 180.189) while the tolerance for tau-fluvalinate (fluvalinate isomers are not differentiated) is 20 µg/kg in honey (CFR Title 40 part 180.427). No extracted honey exceeded the coumaphos or fluvalinate tolerances. One sample of brood comb honey, which is the honey consumed by overwintering honey bees, exceeded the human tolerance for fluvalinate while 4 samples exceeded the tolerance for coumaphos (Table 1).

There are no honey tolerances for any of the other pesticides observed. These compounds have no labelled use within the bee hive, and therefore any amount observed would be considered in violation of the regulations. These compounds were probably brought into the colony by foraging bees. While the sample procedure in this study does not distinguish between nectar contaminated with pesticide residues at the nectar source (plants) and nectar/honey contaminated in the hive due to migration of residues from other matrices, recent research shows that it is possible for foraging honey bees to bring back pesticide contaminated pollen (Mullin *et al.,* 2010) thus providing some evidence in favour of the possibility of pesticide contaminated nectar from plants.

While our analytical techniques did not allow for the quantification of thymol or dichlorobenzene, the frequency of detection was 12% and 47%, respectively. Thymol was only observed in honey from brood comb, whereas dichlorobenzene was observed in honey from supers and from brood comb. Ebert *et al.* (2007) reported thymol to have very low adult bee toxicity. Though the effect of thymol on larvae was not tested, and it is possible that larvae could be more sensitive than adults, adults would be exposed to higher concentrations than the larvae and larval exposure would be mediated through the workers. Dichlorobenzene was detected in nearly half of all samples and 38% of the honey samples from supers. These results for honey from extracted supers are similar to those reported by Bogdanov *et al.* (2004), who found dichlorobenzene residues in 30% Swiss honey samples.

Pesticide residues in honey, whilst low in comparison to other substrates in the hive, contribute to the overall pesticide exposure of honey bees. Honey, along with pollen, is a component of brood food and royal jelly, so pesticide exposure from food may be sufficient to harm larvae either through single or synergistic exposure. Wax may also be a significant source of pesticide exposure for larvae. Further work to determine the impact of the various sources of pesticide residue, as well as the impact of sublethal single exposures and possible interactions among the pesticide residues, is needed.

# References

AL-RIFAI, J; AKEEL, N (1997) Determination of pesticide residues in imported and locally produced honey in Jordan. *Journal of Apicultural Research*, 36(3/4): 155-161.

ALAUX, C; BRUNET, J-L; DUSSAUBAT, D; MONDET, F; TCHAMITCHAN, S; COUSIN, M; BRILLARD, J; BALDY, A; BELZUNCES, L P; LE CONTE, Y (2010) Interactions between *Nosema* microspores and a neonicotinoid weaken honey bees (*Apis mellifera*). *Environmental Microbiology*, 12(3): 774-782. http://dx.doi.org/10.1111/j.1462-2920.2009.02123.x

ALIOUANE, Y; EL HASSANI, A K; GARY, V; ARMENGAUD, C; LAMBIN, M (2009) Subchronic exposure of honey bees to sublethal doses of pesticides: effects on behaviour. *Environmental Toxicology and Chemistry*, 28(1): 113-122.

ANASTASSIADES, M; LEHOTAY, S; STAJNBAHER, D; SCHENK, F (2003) Fast and easy multiresidue method employing acetonitrile extraction/ partitioning and "dispersive solid phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International*, 86: 412-431.

ATKINS, E L (1992) Injury to honey bees by poisoning. In *J M Graham (Ed.). The hive and the honey bee.* Dadant & Sons; Hamilton, IL, USA. pp 1153-1208.

AUPINEL, P; FORTINI, D; MICHAUD, B; MAROLLEAU, F; TASEI, J-N; ODOUX, J-F (2007) Toxicity of dimethoate and fenoxycarb to honey bee brood (*Apis mellifera*), using a new *in vitro* standardized feeding method. *Pest Management Science*, 63: 1090-1064. http://dx.doi.org/10.1002/ps.1446

AUSTRALIAN PESTICIDE AND VETERINARY AUTHORITY (APaVM) (2004) *Evaluation of the new active Boscalid in the product Filan Fungicide*. APaVM; Kingston, AU. 41 pp.

ÁVILA ALEXANDRE, C; RUIZ-ATIENZA-RUIZ, L; BALAGUER PASCUAL, V (1990) Pesticide residues in honey *Vida Apícola*, 44: 14-17.

BEVK, D; KRALJ, J; COKL, A (2012) Coumaphos affects food transfer between workers of honey bee *Apis mellifera*. *Apidologie*, 43: 465 -470. http://dx.doi.org/10.1007/s13592-011-0113-x

BLASCO, C; FERNÁNDEZ, M; PENA, A; LINO, M$^{A}$C; SILVEIRA, I; FONT, G; PICÓ, Y (2003) Assessment of pesticide residues in honey samples from Portugal and Spain. *Journal of Agricultural and Food Chemistry*, 51(27): 8132-8238. http://dx.doi.org/10.1021/jf034870m.

BOGDANOV, S (2006) Contaminants of bee products. *Apidologie*, 37: 1-18. http://dx.doi.org/10.1051/apido:2005043

BOGDANOV, S; IMDORF, A; KILCHENMANN, V (1998a) Residues in wax and honey after Apilife VAR treatment. *Apidologie*, 29: 513-524.

BOGDANOV, S; KILCHENMANN, V; IMDORF, A (1998b) Acaricide residues in some bee products. *Journal of Apicultural Research*, 37(2): 57-67.

BOGDANOV, S; KILCHENMANN, V; SEILER, K; PFEFFERLI, H; FREY, T H; ROUS, B; WENIK, P; NOSER J (2004) Residues of *para*-dichlorobenzene in honey and bees wax. *Journal of Apicultural Research*, 43(1): 14-16.

BROMENSHENK, J J; HENDERSON, C B; WICK, C H; STANFORD, M F; ZULICH, A W; JABBOUR, R E; DESHPANDE, S V; MCCUBBIN, P E; SECCOMB, R A; WELCH, P M; WILLIAMS, T; FIRTH, D R; SKOWRONSKI, E; LEHMANN, M M; BILIMORIA, S L; GRESS, J; WANNER, K W; CRAMER, R A Jr (2010) Iridovirus and microsporidian linked to honey bee decline. *PLoS ONE*, 5(10): e13181. http://dx.doi.org/10.1371/journal.pone.0013181

CHAUZAT, M-P; MARTEL, A-C; ZEGGANE, S; DRAJNUDEL, P; SCHURR, F; CLÉMENT, M-C; RIBIÈRE-CHABERT, M; AUBERT M; FAUCON, J-P (2010) A case control study and a survey on mortalities of honey bee colonies (*Apis mellifera*) in France during the winter of 2005-6. *Journal of Apicultural Research*, 49(1): 40-51. http://dx.doi.org/10.3896/IBRA.1.49.1.06

CHEN, Y P; HUANG, Z Y (2010). *Nosema ceranae*, a newly identified pathogen of *Apis mellifera* in the USA and Asia. *Apidologie*, 41(3): 364-374. http://dx.doi.org/10.1051/apido/2010021

CHOUDHARY, A; SHARMA, D C (2008) Pesticide residues in honey samples from Himachal Pradesh (India). *Bulletin of Environmental Contamination and Toxicology*, 80(5): 417-422. http://dx.doi.org/10.1007/s00128-009-9426-5

CFR (Code of Federal Regulations) Title 40 Part 180. United States Government (2011). http://www.gpo.gov/fdsys/pkg/CFR-2011-title40-vol24/pdf/CFR-2011-title40-vol24-part180.pdf

COLIN, M E; BONMATIN, J M; MONIEAU, I; GAIMON, C; BRUN, S; VERMANDERE, J P (2004) A method to quantify and analyse the foraging activity of honey bees; relevance to the sublethal effects induced by systemic insecticides. *Archives of Environmental Contamination and Toxicology*, 47: 387-395. http://dx.doi.org/10.1007/s00244-004-3052-y

CONRAD, R (2009) Colony Collapse Disorder Part I: a sign of things to come. *Bee Culture*, 137(6): 1-7.

CONRAD, R (2009) Colony Collapse Disorder Part II: faulty assumptions. *Bee Culture*, 137(8): 1-4.

COX-FOSTER, D L; CONLAN, S; HOLMES, E C; PALACIOS, G; EVANS, J D; MORAN, N A; QUAN, P-L; BRIESE, T; HORNIG, M; GEISER, D M; MARTINSON, V; VANENGELSDORP, D; KALKSTEIN, A L; DRYSDALE, A; HUI, J; ZHAI, J; CUI, L; HUTCHINSON, S K; SIMONS, J G; EGHOLM, M; PETTIS, J S; LIPKIN, W I (2007) A metagenomic survey of microbes in honey bee colony collapse disorder. *Science*, 318: 283-237. http://dx.doi.org/10.1126/science.1146498

DE ALMEIDA ROSSI, C; ROAT, T C; TAVERES, D A; CINTRA-SOCOLOWSKI, P; MALASPINA, O (2013) Effects of sublethal doses of imidacloprid in Malphighian tubules of Africanized *Apis mellifera* (Hymenoptera, Apidae). *Microscopy Research and Techniques*, 76: 552-558. http://dx.doi.org/10.1002/jemt22199

DECOURTYE, A; LACASSIE, E; PHAM-DELEGUE, M-H (2003) Learning performances of honey bees (*Apis mellifera* L) are differentially affected by imidacloprid according to season. *Pest Management Science*, 59: 269-278. http://dx.doi.org/10.1002/ps.631

DECOURTYE, A; DEVILLERS, J; GENECQUE, E; LE MENACH, K; BUDZINSKI, H; CLUZEAU, S; PHAM-DELEGUE, M-H (2005) Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honey bee *Apis mellifera*. *Archives of Environmental Contamination and Toxicology*, 48: 242-250. http://dx.doi.org/10.1007/s00244-003-0262-7

EBERT, T A; KEVAN, P G; BISHOP, B L; KEVAN, S D; DOWNER, R A (2007) Oral toxicity of essential oils and organic acids to honey bees (*Apis mellifera*). *Journal of Apicultural Research*, 46(4): 220-224.

FERNÁNDEZ-MUIÑO, M A; SANCHO, M T; MUNIATEGUI, S; HUIDOBRO, J F; SIMAL-LOZANO, J (1995) Nonacaricide pesticide residues in honey: analytical methods and levels found. *Journal of Food Protection*, 58(11): 1271-1274.

JOHNSON, R M; POLLOCK, H S; BERENBAUM, M R (2009) Synergistic interactions between in-hive acaricides in *Apis mellifera*. *Journal of Economic Entomology*, 102(2): 474-479. http://dx.doi.org/10.1603/029.102.0202

JOHNSON, R M; ELLIS, M D; MULLIN, C A; FRAZIER, M (2010) Pesticides and honey bee toxicity - USA. *Apidologie*, 41: 312-331. http://dx.doi.org/10.1051/apido/2010018

HALLENBECK, W H; CUNNINGHAM BURNS, K M (1985) *Pesticides and human health*. Springer Verlag; New York, NY, USA. 166 pp.

HARDING, W C (1979) Pesticide profiles. Part one: insecticides and acaricides. *Bulletin*, 267. Cooperative Extension Service. University of Maryland.

HATJINA, F; PAPEFTHIMIOU, C; CHARISTOS, L; DOGAROGLU, T; BOUGA, M; EMMANOUIL, C; ARNALD, G (2013) Sublethal doses of imidacloprid decrease size of hypopharyngeal glands and respiratory rhythm of honey bees *in vivo*. *Apidologie*, 44: 467-480. http://dx.doi.org/10.1007/s13592-013-0199-4

HEYLEN, K; GOBIN, B; ARCKENS, L; HUYBRECHTS, R; BILLEN, J (2011) The effects of four crop protection products on the morphology and ultrastructure of the hypopharyngeal gland of the European honey bee, *Apis mellifera. Apidologie*, 42: 103-116. http://dx.doi.org/10.1051/apidol/2010043

KOCHANSKY, J; WILZER, K; FELDLAUFER, M (2001) Comparison of the transfer of coumaphos from bees wax into syrup and honey. *Apidologie*, 32: 119-125.

KROL, W J; EITZER, B D; MATTINA, M I; ARSENAULT, T (2010) Pesticide residues in produce sold in Connecticut 2007. *The Connecticut Agricultural Experiment Station Bulletin No. 1024*, New Haven, CT, USA. 1-16.

LODESANI, M; PELLACANI, A; BERGOMI, S; CARPANA, E; RABITTI, T; LASAGNI, P (1992) Residue determination for some products used against *Varroa* infestation in bees. *Apidologie*, 23: 257-272.

MARTELL, A-C; ZEGGANE, S; AURIERES, C; DRAHNUDEL, P; FAUCON, J-P; AUBERT, M (2007) Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar® or Asuntol® 50. *Apidologie*, 38(6): 534-544. http://dx.doi.org/10.1051/apido:2007038

MEISTER, R T (1992) *Farm chemicals handbook '92*. Meister Publishing Company; Willoughby, Ohio, USA. 516 pp.

MULLIN, C A; FRAZIER, M; FRAZIER, J L; ASHCRAFT, S; SIMONDS, R; VANENGELSDORP, D; PETTIS, J S (2010) High levels of acaricides and agrochemicals in North American apiaries: implications for honey bee health. *PLoS ONE*, 5(3): e9754. http://dx.doi.org/10.1371.journal.pone.0009754

NEUMANN, P, CARRECK, N L (2010) Honey bee colony losses. *Journal of Apicultural Research*, 49(1): 1-6. http://dx.doi.org/10.3896/IBRA.1.49.1.01

PALMER, M J; MOFFAT, C; SARANZEWA, N; HARVEY, J; WRIGHT, G A (2013) Cholinergic pesticides cause mushroom body neuronal inactivation in honey bees. *Nature Communications*, 4: 1635. http://dx.doi.org/10.1038/ncomms2648

ROAT, T C; CARVALHO, S M; NOCELLI, R C F; SILVA-ZACARIN, E C M; PALMA, M S; MALASPINA, O (2013) Effects of sublethal dose of fipronil on neuron metabolic activity of Africanized honey bees. *Archives of Environmental Contamination and Toxicology*, 64: 456 -466. http://dx.doi.org/10.1007/s00244-012-9849-1

SARFRAZ KHAN, M; KUMARI, B; ROHILLA, H R; KAUSHIK, H D; ARORA, R K (2004) Analysis of insecticide residues in honeys from apiary (*Apis mellifera*) and wild honey bee (*Apis dorsata* and *Apis florea*) colonies in India. *Journal of Apicultural Research*, 43(3): 79-82.

SMODIS SKERL, M I; KMECL, V; GREGORC, A (2010) Exposure to pesticides at sublethal level and their distribution within a honey bee (*Apis mellifera*) colony. *Bulletin of Environmental Contamination and Toxicology*, 85: 125-128. http://dx.doi.org/10.1007/s00128-009-9762-0

SPLEEN, A M; LENGERICH, E J; RENNICH, K; CARON, D; ROSE, R; PETTIS, J S; HENSON, M; WILKES, J T; WILSON, M; STITZINGER, J; LEE, K; ANDREE, M; SNYDER, R; VANENGELSDORP, D (2013) A national survey of managed honey bee 2011-2012 winter colony losses in the United States: results from the Bee Informed Partnership. *Journal of Apicultural Research*, 52(2): 44-53. http://dx.doi.org/10.3896/IBRA.1.52.2.07

STEINHAUER, N A; RENNICH, K; WILSON, M E; CARON, D M; LENGERICH, E J; PETTIS, J S; ROSE, R; SKINNER, J A; TARPY, D R; WILKES, J T; VANENGELSDORP, D (2014) A national survey of managed honey bee 2012-2013 annual colony losses in the USA: results from the Bee Informed Partnership. *Journal of Apicultural Research*, 53(1): 1-18. http://dx.doi.org/10.3896/IBRA.1.53.1.01

TREMOLADA, P; BERNARDINELLI, I; COLOMBO, M; SPREAFICO, M; VIGHI, M (2004) Coumaphos distribution in the hive ecosystem: case study for modelling applications. *Ecotoxicology*, 13(6): 589-601. http://dx.doi.org/10.1023/B:ECTX.0000037193.28684.05

VANENGELSDORP, D; EVANS, J D; SAEGERMAN, C; MULLIN, C; HAUBRUGE, E; NGUYEN, B K; FRAZIER, M; FRAZIER, J; COX-FOSTER, D; CHEN, Y; UNDERWOOD, R; TARPY, D R; PETTIS, J S (2009). Colony Collapse Disorder: a descriptive study. *PLoS ONE*, 4(8): e6481. http://dx.doi.org/10.1371/journal.pone.0006481

VANENGELSDORP, D; HAYES, J; UNDERWOOD, R; PETTIS, J S (2010) A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. *Journal of Apicultural Research*, 49(1): 7-14. http://dx.doi.org/10.1371/journal.pone.0004071

VANENGELSDORP, D; HAYES, J; UNDERWOOD, R M; CARON, D; PETTIS, J S (2011) A survey of managed honey bee colony losses in the USA, fall 2009 to winter 2010. *Journal of Apicultural Research*, 50 (1): 1–10. http://dx.doi.org/10.3896/IBRA.1.50.1.01

VANENGELSDORP, D; CARON, D; HAYES, J; UNDERWOOD, R; HENSON, M; RENNICH, K; SPLEEN, A; ANDREE, M; SNYDER, R; LEE, K; ROCCASECCA, K; WILSON, M; WILKES, J; LENGERICH, E; PETTIS, J S (2012) A national survey of managed honey bee 2010-11 winter colony losses in the USA: results from the Bee Informed Partnership. *Journal of Apicultural Research*, 51(1): 115–124. http://dx.doi.org/10.3896/IBRA.1.51.1.14

WALLNER, K (1995) The use of varroacides and their influence of the quality of bee products. *American Bee Journal*, 155(12): 817-821.

WALLNER, K (1999) Varroacides and their residues in bee products. *Apidologie,* 30: 235-248.

WILLIAMSON, S M; WRIGHT, G A (2013) Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honey bees. *Journal of Experimental Biology*, 216: 1799-1807. http://dx.doi.org/10.1242/jeb.083931