AMERICAN SCIENCE TEAM FINDS 118 DIFFERENT AGRICULTURAL PESTICIDES IN U.S. BEEHIVES

Honey bees across North America are extensively exposed to multiple pesticides

" *Brood nest wax and foundations, beebread and trapped pollen, and adult bees and brood comprising 749 samples contained* ***118*** *different pesticides and metabolites, 4894 total residues of which 748 were systemics, and averaged 6.5 detections per sample. In the 259 wax samples (*[***Table 1***]*) 87 pesticides and metabolites were found with up to 39 different detections in a single sample, averaging 8 different pesticide residues each. In the 350 pollen samples analyzed (*[***Table 2***]*), 98 pesticides and degradates were identified, with up to 31 different pesticides found in a single sample, and samples averaged 7.1 different pesticide residues each. The analysis of bees resulted in fewer detections (*[***Table 3***]*), and averaged 2.5 residues per each of the 140 samples, with a maximum of 25 in one sample. Only one of the wax, three pollen and 12 bee samples had no detectable pesticides.*

We have found unprecedented levels of miticides and agricultural pesticides in honey bee colonies from across the US and one Canadian province. While these samples were not part of a full-scale landscape or grower-level survey, the data contained here is the largest sampling of pesticide residues in N. American bee colonies or worldwide to date, and represents a cost of nearly $175,000 for the analyses alone. We attempt here to draw trends from these data to indicate both potential risks for bee health as well as justifying the need for greater investments in monitoring pesticide residues in the future.
While a slightly larger number of pesticides are found by including materials associated with beekeeping such as corn syrup, pollen substitute, royal jelly, honey and floral nectars, the trends are well represented by the hive contents of pollen, wax, and bees. A comparison of **Tables 1, 2, 3, 4** with **Tables S1** and **S2** indicates that a number of currently used pesticides (e.g. alachlor, dimethoate) were not found in any samples, and that some of the most environmentally persistent pesticides banned from use in the last 10 years (e.g. aldrin, endrin) also do not appear.

High levels of multiple pesticides in bee-collected pollen

High levels of fluvalinate and coumaphos are co-occuring with lower but significant levels of 98 other insecticides, fungicides and herbicides in pollen. Most noteworthy were the very high levels of the fungicide chlorothalonil in pollen and wax (**Tables 1, 2, 4**) as well as ppm levels of the insecticides aldicarb, carbaryl, chlorpyrifos and imidacloprid, fungicides boscalid, captan and myclobutanil, and herbicide pendimethalin. With an average of 7 pesticides in a pollen sample, the potential for multiple pesticide interactions affecting bee health seems likely. Ten pesticides were found in pollen at greater than one tenth the bee LD_{50} level indicating that sublethal effects of these toxicants alone are highly likely. European researchers have noted fewer and usually lower levels of pesticides in pollen samples, although high detections of particularly carbamates and pyrethroids have been reported [8], [32].

As pollen is the main protein source for developing brood and is intimately involved in development of the hypopharyngeal glands of nurse bees [33], which in turn affects their ability to rear brood, surviving on pollen with an average of 7 different pesticides seems likely to have consequences. Requirements for protein at the colony level vary markedly over the growing season, and the ability of the hive as a superorganism to respond to these changing needs may be compromised by the plethora of pesticides we documented in pollen. Given the critical role played by pollen in bee nutrition and colony dynamics, the complete lack of understanding of chemical biotransformations of pesticides in stored beebread compels a need for additional work.
It is well documented that neonicotinoid pesticides occur in pollen at levels that affect the learning ability of bees fed such pollen [8]–[11], but adding other fungicides or pesticides into this mix has yet to be considered. Bees have genes for specific types of nicotinic acetylcholine receptors [34], and therein may lie the special sensitivity they have to neonicotinoids, but behavioral outcomes of selective actions at these molecular targets has yet to be investigated. Growers of many bee-pollinated crops routinely apply fungicides during bloom, while pollinators are present [35] as there are currently no label restrictions for this action. Thus it may not be

surprising that fungicides account for most of the pesticide content of pollen (**Figure 2a**). Kubik et al. [36] noted high residues of the fungicides vinclozolin and iprodione up to 32 and 5.5 ppm respectively, in beebread. Chlorothalonil is the most frequent detection in pollen and wax after fluvalinate and coumaphos, and all three coincide in 47% of our pollen and wax samples. Chlorothalonil is a highly reactive, widely-used, broad-spectrum fungicide that promotes oxygen stress [37] and is overtly toxic to fish and other aquatics at ppb levels [38]. We found chlorothalonil to be a marker for entombing behavior in bee colonies associated with poor health [24], and it was suggested that entombing may be a new defensive behavior of bees faced with large amounts of potentially toxic food stores. Pollen appears to be the source of chlorothalonil residues in wax, as the pollen levels are higher and correlative of the levels in wax from the same colonies (**Figure 2b**). Chlorothalonil content in beebread is expectedly driven by bees foraging on this non-systemic fungicide either directly by picking up pollen-sized particle formulations or through their presence where pollen, nectar, or water is collected. Some fungicides have shown direct toxicity to honey or solitary bees at field use rates [39], but consequences of chlorothalonil in pollen and beebread fed to bee brood and adults alone or in conjunction with other pesticides remains to be determined.

High levels of miticides in comb wax

Beeswax remains the ultimate sink from the long-term use of the miticides fluvalinate, coumaphos, amitraz (**Table 4**) and bromopropylate [40], reaching 204, 94, 46 and 135 ppm, respectively. Colony residue levels of these miticides, after their in-hive application, have been shown to increase from honey to pollen to beeswax [16], [40]–[45]. Beeswax is the resource of the hive that is least renewable and is thus where persistent pesticides can provide a "toxic-house" syndrome for the bees. The uniform high levels of these miticides present in foundation (**Table 5**) is particularly disturbing, since replacement of comb is currently recommended to reduce pesticide contaminants. The broad contamination of European foundation with especially miticides has been reviewed previously [43]. Fluvalinate residues in beeswax best correlated with the French bee winter kill of 1999–2000 [5], although disease factors were more emphasized in the report. Out of the surveyed apiaries suffering severe bee mortality, 79% of their wax samples contained this miticide in contrast to 76% harboring one or more serious diseases.

Almost all wax and pollen samples (98.4%) contained two or more pesticide residues, of which greater than 83% were fluvalinate and coumaphos (**Table 4**). Clearly, substantial residues of these bee-toxic pyrethroid and organophosphate compounds prevailed together in most beehives sampled. Chronic exposures to high levels of these persistent neurotoxicants elicits both acute and sublethal reductions in honey bee fitness, especially queens [46], [47], and they can interact synergistically on bee mortality [48]. Our work does not directly associate these miticides with CCD, although higher coumaphos levels may actually benefit the colony, possibly via mite control [18].

Almost 60% of our pollen and wax samples, in contrast to 11% of bee samples, contained at least one of 43 systemic pesticides, 57% in combination with a pyrethroid. Substantial amounts of potentially synergistic fungicides such as cyprodinil, fenbuconazole, myclobutanil and propiconazole were also found. Fungicides generally have low bee toxicity by themselves, but exceptions with captan and the ergosterol biosynthesis inhibitor (EBI) propiconazole have been reported [39]. The latter as well as myclobutanil are potent synergists for the pyrethroid cyhalothrin [49]. The frequent coincidence in pollen of high levels of the non-systemic fungicide chlorothalonil with lower levels of systemic pesticides including fungicides is another probable synergistic combination that needs further exploration concerning bee decline.

Lower levels of pesticides in bees

Bees generally have lower pesticide residues than pollen [**Table 4**, 32]. Samples taken from unhealthy CCD-associated colonies were from live bees at the time of collection and represent house bees or residual foragers. These most likely were newly emerged bees, since older bees are typically missing from fully collapsed hives. Fluvalinate exceeded coumaphos residues in these bees, but even the highest detection of 6 ppm (**Table 4**) is less than half the LD_{50}, and by itself could account for only a low death level. We found chlorothalonil at 100-fold lower concentrations in bees compared to pollen or wax indicating its rapid biotransformation to undetected or excreted metabolites (**Table 4**).

Biotransformations and rapid excretion may also explain the general lack of systemic pesticide residues in bees. **"**

The link to the full paper is here:
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0009754