Chlorinated Hydrocarbon Insecticide Residues in Tennessee Honey and Beeswax

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Abstract

Several samples of commercial grade honey collected from different parts of Tennessee during the summer of 1973 were analyzed for chlorinated hydrocarbon insecticide (CHI) residues. A "Modified Mill's Procedure" was used to cleanup the samples prior to gas chromatographic analysis using electron capture (EC) detection. The presence of CHI residues was confirmed by analysis on three different columns of widely varying polarity. Most of the samples contained CHI residues at 0.01-0.30 parts per billion (ppb) level. Beeswax produced during the same season contained several times higher levels of the residue than the honey samples. Recoveries of CHI residues varied from 81-95 percent by the procedure employed.

Introduction

Samples of honey and beeswax produced by beekeepers from east, middle, and west Tennessee during the summer of 1973 were analyzed for chlorinated hydrocarbon insecticides to determine (i) the residue levels, and (ii) if any residues found resulted from the use of such insecticides in the beehives or in the vicinity. A questionnaire was given to each participating beekeeper to gather information about the origin of the samples, principal source of nectar, insecticides used in the beehives and in the immediate vicinity for control operations.

Analysis of Honey

Twenty grams of the honey were weighed into a 250 ml beaker and mixed with 50 ml of distilled water. One hundred ml of acetonitrile was added to the mixture and stirred well. The mixture was transferred into a one-liter separatory funnel, the beaker washed with hexane and washings were added to the separatory funnel. The funnel was shaken vigorously for two minutes and allowed to stand until the phases separated. The acetonitrile-water phase was drained into another separatory funnel and hexane (50 ml) and

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water (600 ml) were added to it and shaken gently for one minute. An emulsion always formed in the hexane layer which was broken up easily by the addition of anhydrous sodium sulfate. The hexane layer was mixed with the first extract and the acetonitrile-water layer was extracted again with a fresh 50 ml portion of hexane. The combined hexane extracts were washed three times with 25 ml portions of water, filtered through a layer of anhydrous sodium sulfate and evaporated to about 2 ml in a rotary evaporator under vacuum.

The concentrated residue was pipetted into a glass column (15 x 100 mm) which contained 6 cm of Florisil (Fisher) topped with 1 cm of anhydrous sodium sulfate. The Florisil and sodium sulfate used in this study were heated at 600°C for 24 hours and allowed to cool in a desiccator. The residues were eluted with 100 ml of 1% v/v ethyl ether-hexane solution and collected in a 250 ml round bottom flask. The eluate was subsequently evaporated to less than 1 ml in a rotary evaporator under vacuum and diluted to exactly 1 ml with hexane.

All the solvents were Nanograde quality and the glassware was heat-treated before use at 200°C as described by BEVENUE et al. (1).

A Varian 1800 gas chromatograph equipped with a tritium EC detector was used for analysis of the concentrate. The three columns used to confirm the presence of CHI residues were

1) 6' x 2 mm glass column packed with 3% OV-1 on 100/120 mesh Gas-Chrom Q (non-polar).
2) 6' x 2 mm glass column packed with 1.5% OV-17 + 1.95% QF-1 on 80/100 mesh Supelcoport (moderately polar).
3) 6' x 2 mm glass column packed with 3% DEGS on 60/80 mesh Gas-Chrom Q (polar).

The temperatures and carrier gas flow rates were adjusted such that a multiple dose standard (MDS) of CHI from Supelco injected into the columns produced well resolved standard chromatograms (Reference) of the various components.

Analysis of Beeswax

Two grams of beeswax cappings were weighed into a 250-ml beaker. Acetonitrile (100 ml) was added to the beaker which was warmed in a water bath (about

60°C) until the beeswax melted. The beaker with contents was then quickly cooled to about -10°C with dry ice, and the contents were filtered through glasswool into a one-liter separatory funnel. Subsequently, the procedures for the extraction and cleanup of beeswax were similar to the analysis of honey except that all solvents and the cleanup column were cooled before use to approximately -5°C.

Results and Discussion

Cleanup of the concentrated extract by MILLS et al. (2) or KADOUM (3) procedures did not produce satisfactory results. Use of 1% v/v ethyl ether in hexane yielded better eluates with sufficiently high recoveries of all the components of the MDS employed. Several fortification studies were carried out in both honey and beeswax samples to establish the recovery of the residues by this procedure. The data are shown in Tables 1 and 2. Figure 1 shows a typical gas chromatogram of a fortified honey sample.

TABLE 1
Recoveries of Chlorinated Hydrocarbon Insecticides Following the Extraction and Cleanup Procedure for Honey

Insecticide	ppb Added	ppb Recovered	*% Recovery
α-BHC	0.02	0.019	93
β-BHC	0.08	0.074	92
Lindane	0.02	0.017	86
Heptachlor	0.02	0.017	84
Aldrin	0.04	0.034	86
Heptachlor epoxide	0.064	0.053	83
p,p'-DDE	0.0152	0.0132	87
Dieldrin	0.096	0.082	86
p,p'-DDT	0.208	0.185	89

* Represents an average of five fortified samples.

TABLE 2
Recoveries of Chlorinated Hydrocarbon Insecticides Following the Extraction and Cleanup Procedure for Beeswax

Insecticide	ppb Added	ppb Recovered	*% Recovery
α-BHC	0.02	0.018	91
β-BHC	0.08	0.076	95
Lindane	0.02	0.019	94
Heptachlor	0.02	0.019	93
Aldrin	0.04	0.032	81
Heptachlor epoxide	0.064	0.053	83
p,p'-DDE	0.152	0.123	81
p,p'-DDD	0.160	0.142	89
o,p'-DDT	0.180	0.171	95
p,p'-DDT	0.280	0.266	95

* Represents an average of three fortified samples.

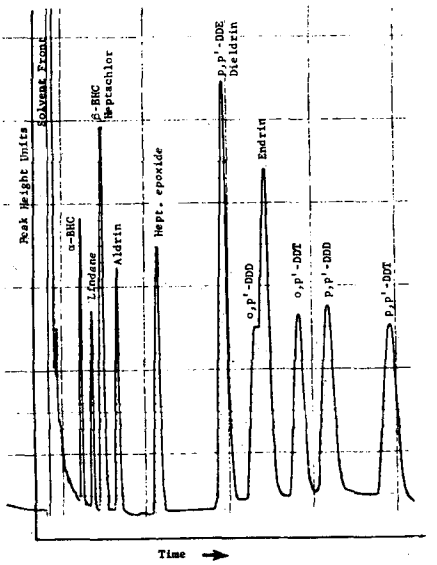

FIGURE 1
Gas Chromatogram (1.5% OV-17 + 1.95% QF-1 Column) of a Residue Honey Sample Fortified with the MDS

Linearity of the detector response to varying amounts of each CHI residue was established using the peak height method. Presence of any residue was confirmed only if it emerged on all three columns and the retention time of the particular component on each of the columns agreed with that of the standard injection.

Tennessee beekeepers submitted 30 honey samples to be analyzed for CHI. Sixteen samples were from east, eight from middle, and six from west Tennessee. The limit of detection was 0.01 ppb for CHI. Twenty-eight of the thirty samples contained detectable CHI, and none was detected in two samples.

Table 3 gives the results of the honey analyses.

TABLE 3
Range in Parts per Billion
of Chlorinated Hydrocarbon Insecticides
in Honey from Tennessee

Insecticide	East	Middle	West
α-BHC	0.03-0.08	0.01-0.08	0.01-0.04
β-BHC	0.03-0.05	0.12	0.29
Lindane	0.01-0.28	0.02-0.38	0.01-0.27
Heptachlor	0.12-0.17	0.12-0.21	0.21-1.07
Aldrin	0.03-0.08	0.16	0.30
Dieldrin	0.05	0.09	--
p,p'-DDE	0.11-0.14	--	--
p,p'-DDT	*--	--	0.24
Number of Samples Assayed	16	8	6

* Limit of detection was 0.01 ppb.

The levels of CHI detected in honey ranged from 0.01 to 0.38 ppb. These results were about the same concentrations as reported by OGATA and BEVENUE (4).

Twenty beeswax (newly-capped comb) samples were analyzed for CHI. Eight samples were from east, six from middle, and six from west Tennessee. Limit of detection was 0.1 ppb. Nineteen beeswax samples contained detectable CHI, and no CHI was detected in one sample.

Table 4 gives the results of the beeswax analyses.

TABLE 4
Range in Parts per Billion
of Chlorinated Hydrocarbon Insecticides
in Beeswax from Tennessee

Insecticide	East	Middle	West
α-BHC	0.54-3.47	0.36-1.41	0.21-1.72
β-BHC	5.26	1.33-2.77	0.68
Lindane	0.63-5.95	0.29-1.74	0.39-0.76
Heptachlor	0.46-5.22	0.70	--
Aldrin	0.93-4.80	1.80-1.86	0.92
Heptachlor Epoxide	1.33	2.23-3.78	7.10
p,p'-DDE	1.05-2.71	--	1.51-3.18
o,p'-DDT	2.61	--	3.83
p,p'-DDT	2.80-39.41	3.65-10.40	1.56-20.56
p,p'-DDD	*--	--	2.51
Number of Samples Assayed	8	6	6

* Limit of detection was 0.1 ppb.

The levels of CHI detected in beeswax ranged from 0.21 to 39.41 ppb. The number of different CHI in a single beeswax sample varied from none to seven.

The source of CHI found in the samples of honey and beeswax probably originated from the dusting and spraying of crops with insecticides. This type of non-specific insecticidal application could easily contaminate nectar, pollen, propolis, and water sources which the bees could take back to their hives. SOLOV'EVA (5) demonstrated that bees carried nectar and pollen contaminated with carbaryl into their hives. MORTON et al. (6) showed that water containing 2,4,5-T was transported by bees and was detected in honey and beeswax produced by the bees. POURTALLIER and TALIERCIO (7) demonstrated that bees could carry on normal functions even though their honey contained parathion, DDT, and toxaphene.

PIEK (8) suggested the synthesis of beeswax originated in the bee oenocytes and fat cells. Bees

which have ingested honey, pollen, and water contaminated with CHI could accumulate CHI in their fat cells resulting in CHI being deposited in the beeswax.

A comparison of the experimental data from east, middle, and west Tennessee revealed no significant difference in the CHI identified in the three sections of the state. Certain CHI were found in the samples analyzed even though these CHI were not used in close proximity to the beehives. This would seem to indicate that these CHI are present in the environment regardless of the use of CHI in the immediate area.

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