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DETERMINATION OF CARBARYL IN HONEYBEES AND POLLEN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Fifty of seventy bee and pollen samples were found to contain methyl parathion and/or carbaryl. One-third of these were randomly analyzed for azinphos methyl; several were positive. Responsibility for bee kills is difficult to determine and cannot be assigned on the basis of suspicion and a positive confirmatory analysis; other possible causes must be excluded. A multi-residue scheme for the above, plus fenvalerate, is presented. High-performance liquid chromatographic determination of carbaryl on a reversed-phase column (C_8-RCSS) with detection by both UV and fluorescence is discussed. The advantages of the latter detection method in this application are illustrated.

INTRODUCTION

Many pesticides have been implicated as causing losses of honeybee colonies due to inadvertent poisoning, or as the result of misuse[1,2]. However, the problem is usually not evident until several days after pesticide application, and frequently not reported for several weeks[3,4]. Pinpointing a culprit and establishing liability is nearly impossible.

The introduction of microencapsulated insecticides caused further difficulties. Slow release capsules are safer to handle and have longer lasting effectiveness, but being 30–50 μm in diameter, they are indistinguishable from pollen grains, and bees pack them into their pollen pockets and return to the hive; plus, the die out symptoms resemble some natural bee viral and/or protazoa infections[5,6]. Microencapsulated methyl parathion, trade name Penncap-M, is used on many field crops and, until the cause was identified, apiaries of over fifty hives were being wiped out. Replacement colonies installed in the hives subsequently perished[7].

A program was initiated to assess the extent of apicultural damage by pesticides, to discriminate between these incidents and natural mortality, and to determine the seriousness of the problems being attributed to the use of Penncap-M. Seventy bee and pollen samples were analyzed for both methyl parathion and carbaryl, at least one of the two usually having been used in the vicinity of each kill. The synthetic

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pyrethroid, fenvalerate, was an additional suspect in eight instances. In addition, analyses for azinphos methyl (guthion) were conducted on nineteen randomly selected samples.

Good analytical methods were available for most of the insecticides included in the study, however, they had to be refined and incorporated into a multi-residue scheme useful for the frequently limited amount of sample available. Of particular note was that while we could measure carbaryl (Sevin) by high-performance liquid chromatography (HPLC) with UV detection at a sensitivity of <0.1 ppm in these matrices, there were many interfering background peaks that made detection significantly below this level impractical.

Many analytical approaches to carbaryl (1-naphthyl-N-methylcarbamate) determination have been reported and reviewed by Pieper[8] and Jones *et al.*[9]. More recent efforts include multi-residue schemes for screening selected commodities such as fruits[10] and ground water[11] for non-specific pesticides contamination, or analytical methods for carbaryl devised for unique matrices such as blood[12] and pineapple[13]. Extensive modification may be necessary to adapt these for determination in other materials. Acquisition of a spectrofluorometric detector enabled us to rerun bee and pollen extracts previously analysed for carbaryl by reversed-phase HPLC coupled with UV detection. This technique proved to be superior to the UV detection of carbaryl previously employed in the development and implementation of our analytical scheme.

EXPERIMENTAL

Sample collection and preparation

Bee samples consisting of 200 dead specimens, and pollen where available, were collected from reported bee kills. The surrounding areas were immediately canvased to identify recent pesticide spray operations and the samples were analyzed for suspect chemicals using our multi-residue scheme. These samples (20 g) were blended with 100 ml acetone for 2 min, then filtered. Filtrate was transferred to a 500-ml separatory funnel, to which was also added 200 ml water, 30 ml of saturated sodium chloride solution and 100 ml of hexanes. The separated organic layer was dried over sodium sulfate and concentrated to *ca.* 2 ml for gas–liquid chromatographic (GLC) determination of methyl parathion, fenvalerate, and guthion (from pollen only).

Aqueous phase was washed three times with 60 ml chloroform, these combined washes were evaporated to dryness, then 15 ml of acetone plus 50 ml of a precipitating solution containing 1.25 g ammonium chloride and 2.5 ml phosphoric acid per liter were added. After filtering, 20 ml of buffer (6.4 g citric acid, 3.5 g boric acid, 13.7 g sodium hydroxide and 2.1 ml phosphoric acid per liter was added to the aqueous acetone extract; final pH 10.2–10.5. The solution was washed with three times with 50 ml of carbon tetrachloride. The combined organic layers were evaporated and the residue either dissolved in methanol for HPLC analysis of carbaryl, or dissolved in acetone for GLC determination of guthion (from bees).

Pollen samples were usually less than 2.0 g and proportional amounts of extracting solvents, etc., were used. Commercial pollen was spiked with carbaryl and used for comparative UV–fluorescence determinations. Check bees were obtained from a healthy commercial hive.

GLC of methyl parathion

Samples were chromatographed on a Tracor Model 222 gas chromatograph; column, 3% OV-1 on 60–80 mesh Gas-Chrom Q, 1.9 m × 6 mm; column temperature, 200°C; inlet temperature, 220°C; detector temperature, 190°C; carrier, nitrogen; flame photometric detector, P filter. Sensitivity <0.05 ppm. Recovery; pollen 86%, bees 106%.

GLC of azinphos methyl

Samples were chromatographed on a Tracor Model 222 gas chromatograph; column, 3% OV-1 on 60–80 mesh Gas-Chrom Q, 1.9 m × 6 mm; column temperaturte, 212°C; inlet temperature, 225°C; detector temperature 205°C; carrier, nitrogen; flame photometric detector, P filter. Sensitivity <0.02 ppm. Recovery; pollen 88%, bees 92%.

GLC of fenvalerate

Samples were chromatographed on a Tracor Model 222 gas chromatograph; column, 5% DC-200 on 80–100 mesh Gas-Chrom Q, 0.7 m × 6 mm; column temperature, 205°C; inlet temperature, 220°C; detector temperature, 295°C; carrier, nitrogen; ^{63}Ni detector. Sensitivity 0.005 ppm. Recovery; bees 90%.

HPLC of carbaryl

Samples were chromatographed using a Tracor 951 pump with Rheodyne 7125 injector (100 μL loop) and a Waters RCSS (radial compression separation system) with a C_8 reversed-phase column (10 cm × 4.6 mm I.D.). The mobile phase was methanol–water (45:55) at 2.0 ml/min; ambient temperature. Retention time for carbaryl was 14.6 min and varied ±0.5 min depending upon ambient temperature(s). Recovery of carbaryl was 81% from bees (0.5 ppm) and 72% from pollen (0.15 ppm). Detectors employed were a Tracor 970A UV–VIS variable wavelength detector at 220 nm for the initial determinations. Subsequently, sample extracts were rechromatographed under identical conditions with detection by a McPherson 750 spectrofluorometer with 750-02 photometer; 288 nm excitation, 342 nm emission, time constant 5 s, photocathode voltage −600 V, xenon lamp current 7 A.

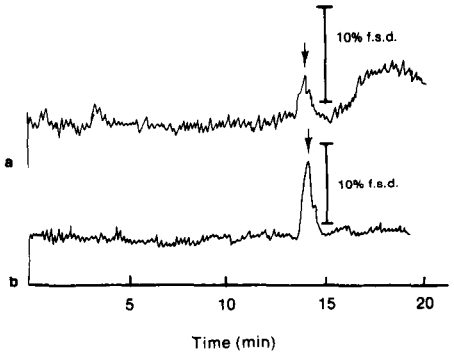
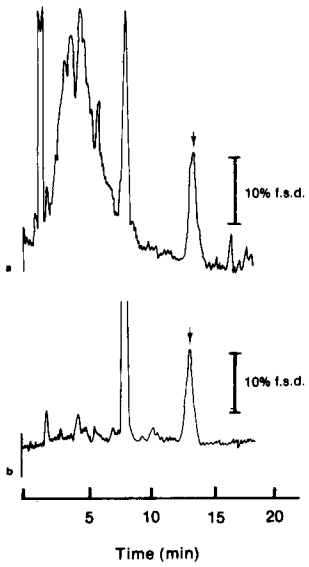


Fig. 1. Chromatograms of carbaryl standard. (a) UV detection at 220 nm (3 ng injected). (b) Fluorescence detection at 288 nm excitation, 342 nm emission (3 ng injected).



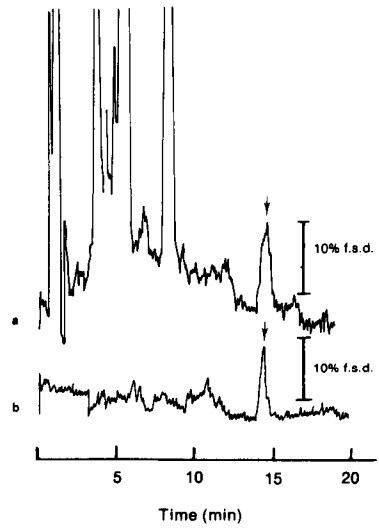


Fig. 2. Chromatograms of carbaryl in bees. (a) UV detection at 220 nm (14 ng injected). (b) Fluorescence detection at 288 nm excitation, 342 nm emission (8 ng injected).

Fig. 3. Chromatograms of carbaryl in pollen. (a) UV detection at 220 nm (8 ng injected). (b) Fluorescence detection at 288 nm excitation, 342 nm emission (4 ng injected).

RESULTS AND DISCUSSION

Fifty of the seventy samples were positive for methyl parathion (>0.1 ppm), fifteen were positive for carbaryl (>0.1 ppm) and five were positive for both. Fenvalerate was not detected in any of the eight samples in which it was suspected, while two of the nineteen randomly selected samples from the groups found to contain either methyl parathion and/or carbaryl, when checked for azinphos methyl, were positive. The instances of multiple lethal residues were frequent, and the residues were not minimal, in fact, they were almost always well above 0.1 ppm. Assigning blame for a bee kill on the basis of applications in the vicinity and analyses for that chemical(s) is clearly not possible. Extensive analyses to eliminate other possible pesticides would be required, and the evidence would still be circumstantial with respect to any particular application event.

The marked improvement of fluorescence detection over UV detection can be seen by inspection of Figs. 1–3. Not only is sensitivity almost double for fluorescence, background noise and the problem of interfering peaks is reduced. Initial reported attempts at employing fluorescence for determination[14] of extracted carbaryl failed because co-extractives were not separated from the compound(s) of interest as they are in HPLC. Separations from plant interferences were effected by partitioning and subsequent chromatography on charcoal-silanized Celite columns by Krause and August prior to HPLC–fluorescence quantitation[15].

However, column cleanup was unnecessary in our multi-residue scheme as many interfering materials in the aqueous phase were eliminated in partition–precip-

itation steps similar to those employed for analysis of other carbamate pesticides — carbofuran[16] and benomyl[17]. This method may be readily setup and employed for carbaryl alone, or indicated fractions may be diverted for GLC determination of other pesticides. This allows for rapid and flexible analytical responses to suspected contamination or exposure.

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