DETERMINATION OF AMINOCARB, FENITROTHION, AND PERMETHRIN RESIDUES IN *B.t.* FORMULATIONS BY GAS LIQUID CHROMATOGRAPHY.

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1. INTRODUCTION

On December 23rd, 1980, eight samples of *Bacillus thuringiensis* formulation, labelled 1,2,4,5,6,7,8 and 10, were received by the Toxic Chemicals Section from C. H. Buchner of CFS Headquarters. They were accompanied by a request that they be analyzed for aminocarb, fenitrothion, and permethrin. The samples arrived in 100-ml., clear, glass bottles with metal screwcaps, and were kept in a cold room at 0.5°C until the analyses were performed.

The origin of these eight samples was unknown to the Toxic Chemicals Section, who were aware only that they were representative of samples taken by C. H. Buchner in July, 1980.

2. ORIGINAL EXTRACTION AND CLEANUP

Two ten-ml. aliquots of Dipel 88, previously analyzed and known to contain no aminocarb, fenitrothion, or permethrin, were fortified by addition of 1 µg of each of these compounds. These two spiked samples, as well as the eight *B.t.* formulations in question, were treated identically.

After allowing the *B.t.* formulations to equilibriate to room temperature, 10.0 mls. of each were pipetted into 100-ml. beakers, to which 20 mls. of ethyl acetate were added. The samples were homogenized for 4 minutes, using a model PT-20 Polytron. The samples were quantitatively transferred into glass centrifuge tubes, and centrifuged for 30 minutes in a Servall Superspeed at 15,000 rpm (27,000 x g). The supernatant was filtered with suction through Whatman GF/A filter paper in a Millipore filtering setup, and the homogenate was washed with ethyl acetate (3 x 2 mls).

The combined ethyl acetate filtrate was flash-evaporated in a Buchler rotary evaporator at 32°C, and a few milliliters of the *B.t.* formulations' co-solvent remained behind. Ten mls. of 10% sulphuric acid were added to the sample, followed by 90 mls. of distilled water. The samples were then transferred to 250-ml. separatory funnels and partitioned with hexane (50, 25, and 25 mls.). After each partitioning, the hexane phase was passed through anhydrous sodium sulphate to remove traces of water.

The hexane phase was flash-evaporated to approximately 2 mls. and further cleaned-up on a 6-gr. 2% deactivated florisil column by eluting the sample with 40% benzene in hexane (100 mls.). The eluate was flash evaporated just to dryness and reconstituted in hexane (2.0 mls.), for gas-liquid chromatographic (GLC) analysis for fenitrothion and permethrin.

The acidic aqueous phase of each sample was neutralized with a saturated sodium carbonate solution. After evolution of CO_2 bubbles stopped, the samples were partitioned with ethyl acetate (50, 25, and 25 mls.). Each ethyl acetate phase was dried by passing through anhydrous sodium sulphate. The combined ethyl acetate phases were flash-evaporated just to dryness, and reconstituted in ethyl acetate (2.0 mls.) for GLC analysis of aminocarb.

3. SIMPLIFIED EXTRACTION OF FORMULATIONS

Preliminary analysis of one of the fortified Dipel 88 samples by GLC indicated quantitative recovery of aminocarb and permethrin. However, analysis of the sample portion containing fenitrothion using the nitrogen-phosphorus detector was unsatisfactory, due to the presence of interfering co-extractives in the hexane phase. Therefore, a simpler and less tedious alternative for extraction was adopted. The new procedures were capable of quantifying residues of all three insecticides at 0.5 ppm-levels as indicated by the good results obtained from extracting and analyzing duplicates of fortified Dipel 88 samples.

Five milliliters of each *B.t.* formulation sample were individually transferred to 15-ml. graduated centrifuge tubes, followed by the addition of ethyl acetate (5 mls.). The tubes were stoppered with teflon-lined screwcaps and shaken vigorously for 3 minutes. They were then centrifuged for 15 minutes at 3000 rpm in an I.E.C. HN-5 centrifuge. Each supernatant was carefully transferred to a clean centrifuge tube using a pasteur pipet, and the volume was adjusted to 10.0 ml. with ethyl acetate. They were further diluted at the ratio of 1 to 1 with ethyl acetate, resulting in a sample size equivalent of 1 ml. *B.t.* formulation per 4 mls. of extract.

The eight *B.t.* formulation extracts thus prepared were analyzed by GLC for aminocarb, fenitrothion, and permethrin.

4. GAS LIQUID-CHROMATOGRAPHIC ANALYSIS

Analysis of the *B.t.* extracts for aminocarb and fenitrothion was performed with a Hewlett-Packard 5710A GLC equipped with a nitrogen-phosphorus detector and a glass column (120 cm x 2 mm i.d., 3% OV-17 on Chrom. W., 80/100 mesh). The carrier gas flow rate (helium) was 30 ml./minute, and the oven temperature was 200°C. The injection port was heated to 250°C and the detector to 300°C. Figures 1 and 2 are typical chromatograms.

Analysis of the samples for permethrin was performed on a Hewlett-Packard 7610A gas chromatograph, using an electron-capture detector with a Ni^{63} radiation source. The column was 120 cm x 2 mm i.d., 3% DC-200 on Gas Chrom. Q, 100/120 mesh, and operated at 220°C. The injection port and detector temperatures were 210°C and 280°C respectively. The carrier gas flow rate (95% argon, 5% methane) was 35 ml./minute. Typical sample chromatograms are given in figures 3 and 4.

Aminocarb and permethrin were not detected in any of the eight *B.t.* formulations extracted by the original method, nor by the simplified method. Fenitrothion was not detected in any of the samples extracted by the simplified method. The samples extracted by the original method were not analyzed for fenitrothion.

5. DISCUSSION AND CONCLUSIONS

When attempting to develop a method for extraction and cleanup of the *B.t.* formulations, initially it was intended to utilize the inherent sensitivity of the Hewlett-Packard nitrogen-phosphorus detector to quantify aminocarb and fenitrothion at levels as low as 0.1 ppm. However, because co-solvents contained in the *B.t.* formulations are various emulsifiers for stabilizing the suspensions, their complete removal was impossible to achieve. Since the cleaned extract interfered with the analysis of fenitrothion, minute amounts of the co-extractives present make analysis of fenitrothion at the 0.1 ppm level impossible. When the simplified extraction procedure was used, the minimum quantifiable limit for all three compounds was 0.5 ppm (0.5 µg per milliliter of formulation).

Aminocarb, fenitrothion, and permethrin were not detected in any of the *B.t.* formulations tested, namely no.s 1,2,4,5,6,7,8, and 10.

Even though our quantification limit for these three insecticides was merely 0.5 ppm, its significance can only be appreciated by relating it to normal spray operations of these chemicals. For example, the application rate in MATACIL® aerial spray was 70 gr. of aminocarb in 1.5 liters of total formulation per hectare. Thus, the concentration of aminocarb is 46.7 gr./ liter spray mix, i.e., 46,700 ppm. Detection of 0.5 ppm aminocarb in the *B.t.* formulations would represent a concentration 1/93,400 of that currently used in spruce budworm spray. Therefore, a method sensitive enough to quantify 0.5 ppm is more than adequate, since any formulation if contaminated at such a level would have no effect on spruce budworm populations at all when aerially applied.

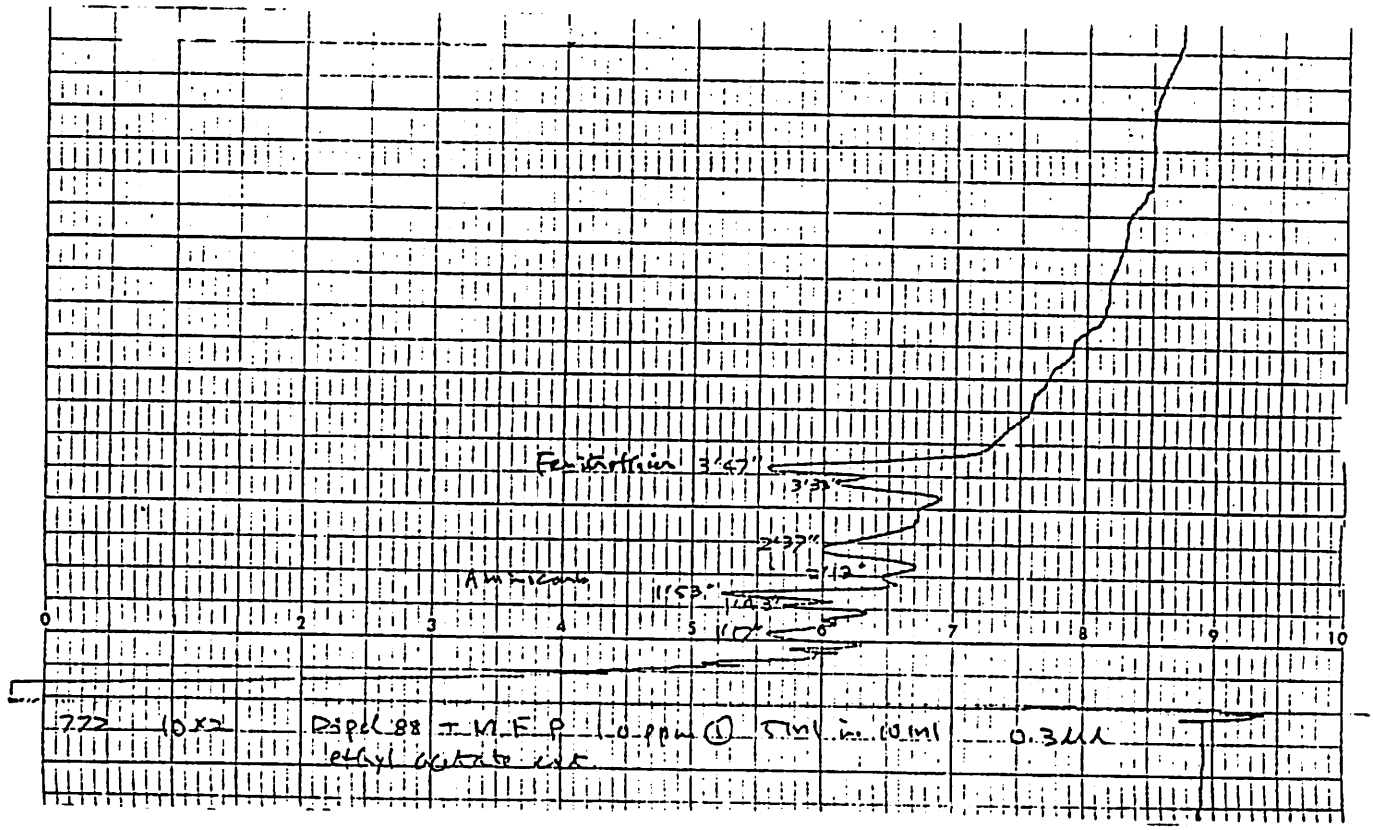

Fig. 1. Dipel 88 fortified with 1.0 ppm aminocarb, fenitrothion, and permethrin. Analysis by HP 5710A with N-P detector.

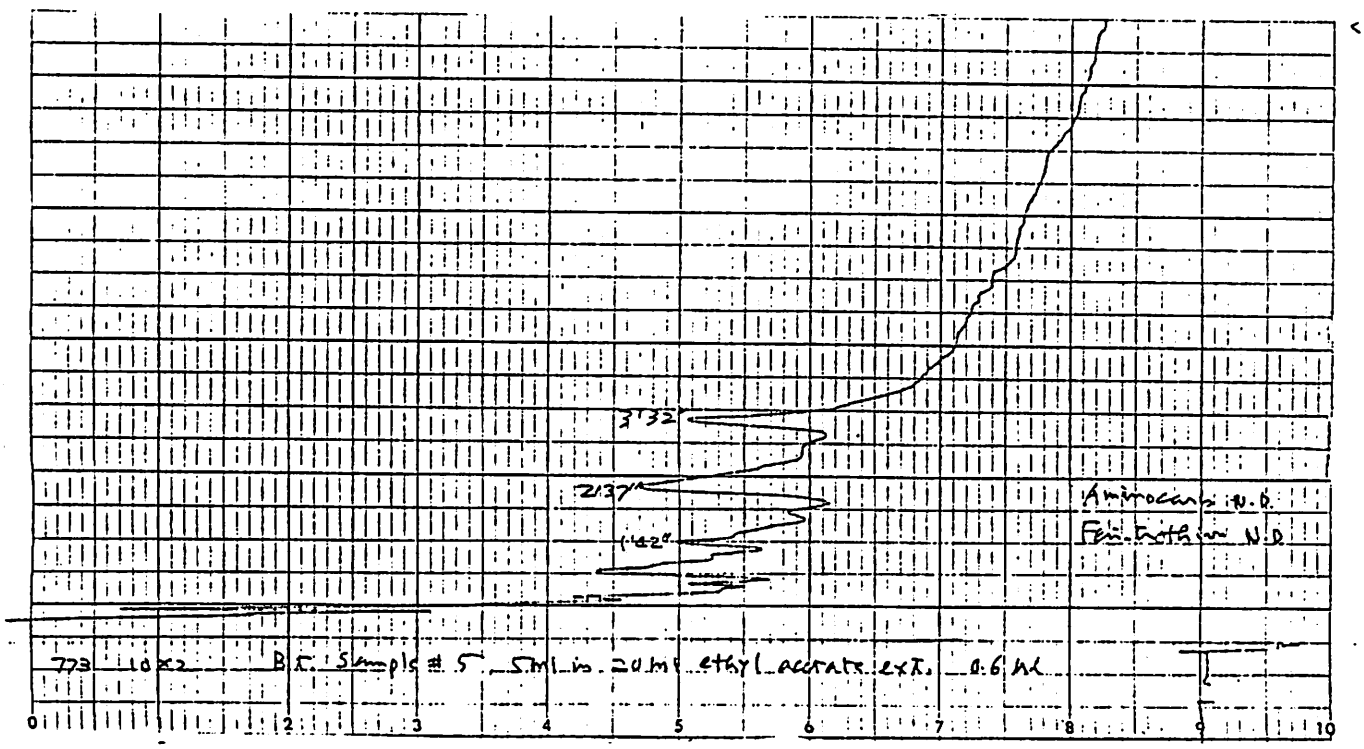

Fig. 2. B.t. sample 5 received from C.H. Buckner. Analysis by HP 5710A with N-P detector.

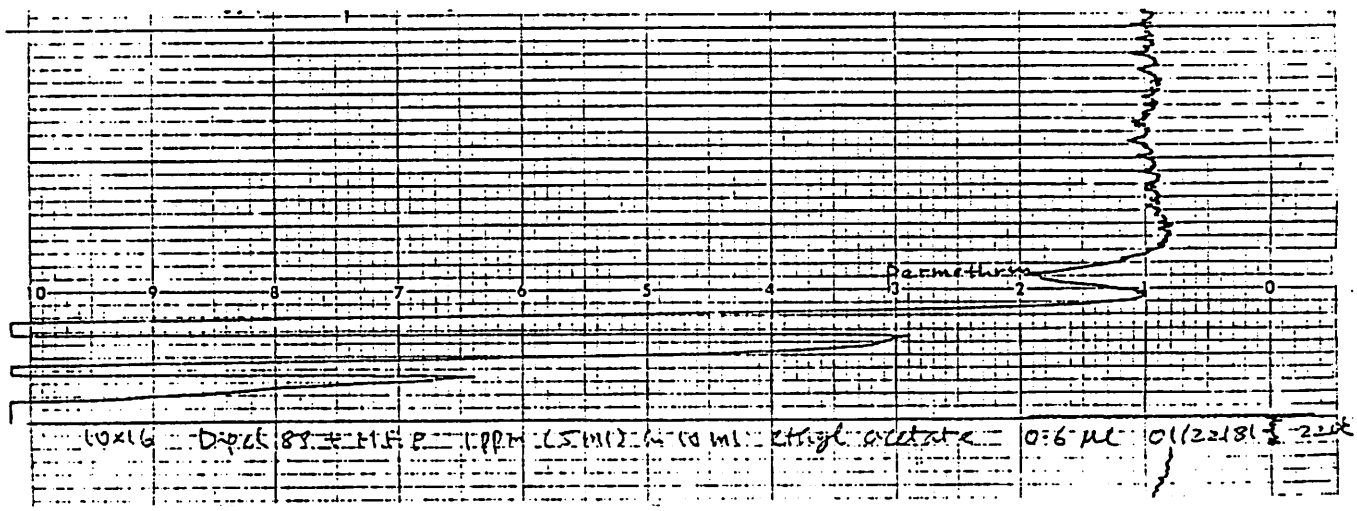

Fig. 3. Dipel 88 fortified with 1.0 ppm aminocarb, fenitrothion, and permethrin. Analysis by HP 7610A with electron-capture detector.

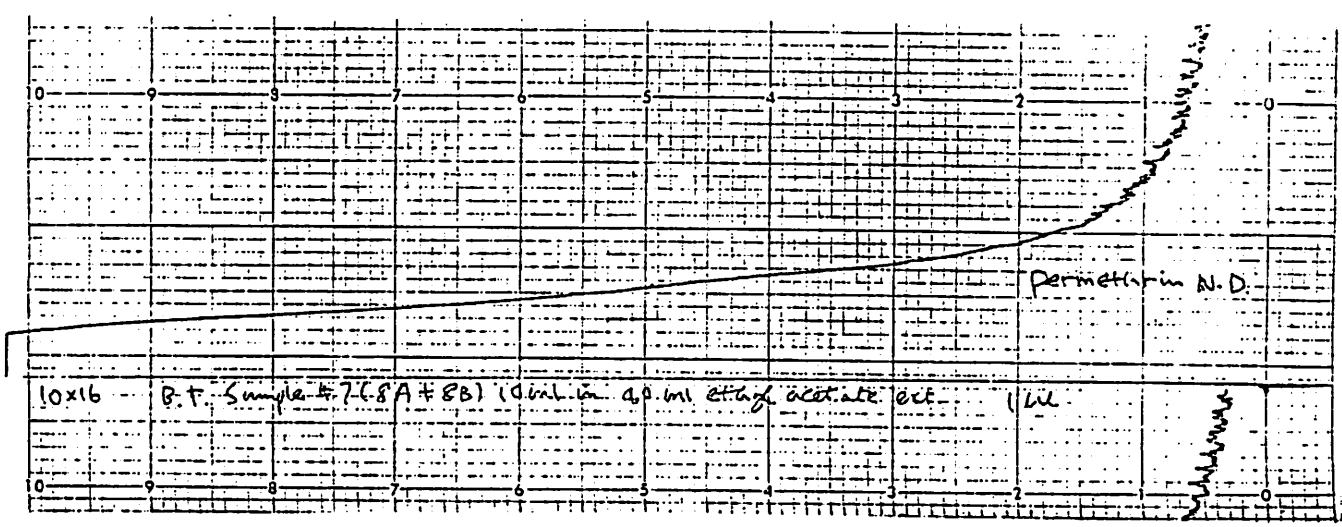

Fig. 4. B.t. sample 7 received from C.H. Buckner. Analysis by HP 7610A with electron-capture detector.