# Identification of Pentachlorobenzonitrile Residues in Field Crops

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We recently received several carrot extracts from another FDA district laboratory for identification of a material present in each of the extracts which had a GLC retention time inconsistent with that of any known pesticide. The material, which was eluted from Florisil with 15% ether in petroleum ether (AOAC, par. 29.015, 1975) exhibited both electron capture and microcoulometric responses (LASKI and LEONE, 1976). This paper reports structural elucidation, GLC characteristics and recovery data for the material.

## MATERIALS AND METHODS

GC-MS-COM System: Pye 104 gas chromatograph interfaced to an AEI MS-30 Double Beam Mass Spectrometer by means of a silicone membrane separator. Data was acquired with an AEI DS-50-DB data system. The mass spectrometer was operated at 70 eV electron energy, 4 Kv accelerating voltage, 300 μA trap current, 250° C source temperature, 3 sec/decade scan speed, 1,000 resolution for low resolution scans and 3,000/3,000 resolution for double beam mass measurements. The gas chromatograph was equipped with a 5 ft x 4 mm i.d. glass column packed with 3% SE-30 on Diatomite, 100-200 mesh, and operated at column and injector temperatures of 190 and 215° C, respectively, and a helium flow rate of 40 ml/min. The membrane separator and transfer lines were heated at 205 and 210° C., respectively.

GLC (for retention time and recovery data): Tracor Model 222 gas chromatograph equipped with a Ni-63 electron capture detector and 6 ft x 4 mm glass columns packed with (A) 10% OV-101 on Chromasorb WHP, 80-100 mesh and (B) a 1:1 mixture of 15% OV-210 and 10% OV-101 on the same support. Column temperature (ca. 200° C) was adjusted to permit elution of pp'-DDT at 3.03 and 3.28 (retention time relative to aldrin) on columns A



Bulletin of Environmental Contamination & Toxicology, Vol. 18, No. 6 © 1977 by Springer-Verlag New York, Inc.

and B, respectively. The detector was adjusted to provide 1/2 full scale deflection for 1 ng heptachlor epoxide.

Sample clean-up: Two of the 15% ether-petroleum ether fractions were combined and streaked on an E. Merck 0.2 mm-thick pre-coated aluminum oxide F-254 neutral (type E) TLC sheet, the sheet having been pre-washed with a solution of n-heptane-acetone (98:2) and activated at 85° C for 15 min. The unknown material was separated by elution with this solution and visualized by spraying the sides of the sheet with a solution of silver nitrate and 2-phenoxyethanol in acetone with subsequent exposure to UV light (MITCHELL, 1961). The material was then scraped off the sheet and eluted with acetone.

## RESULTS AND DISCUSSION

Direct GC-MS analysis of the carrot extract as received indicated a chlorine cluster starting at m/e 273. However, the mass spectrum was quite "dirty" and it was difficult to distinguish between a 4 and 5-chlorine cluster. After clean-up, the low resolution mass spectrum (Fig. 1) indicated a material with a parent ion at m/e 273, 5 chlorine atoms (BEYNON, 1960) and an odd number of nitrogen atoms. The material was thus tentatively identified as pentachlorobenzonitrile ($C_6Cl_5CN$). The structure of this compound was confirmed by obtaining accurate mass measurements and elemental composition data (Table 1) by double beam mass spectrometry. Further structural confirmation was obtained by comparing the GC-MS characteristics of the sample material with that of subsequently obtained authentic material. Quantitation by electron-capture GLC showed that the carrot samples contained approximately 20 ppb pentachlorobenzonitrile, a material which is not an approved pesticide nor an industrial chemical of known usage.

In an attempt to explain how this material was found in the environment, we were at first intrigued by the possibility that it could have been formed by solar irradiation of polychlorinated biphenyls (PCB's) in the presence of $NO_x$ (HUSTER and KORTE, 1974). Further investigation revealed, however, that pentachlorobenzonitrile is a known impurity of the pesti-

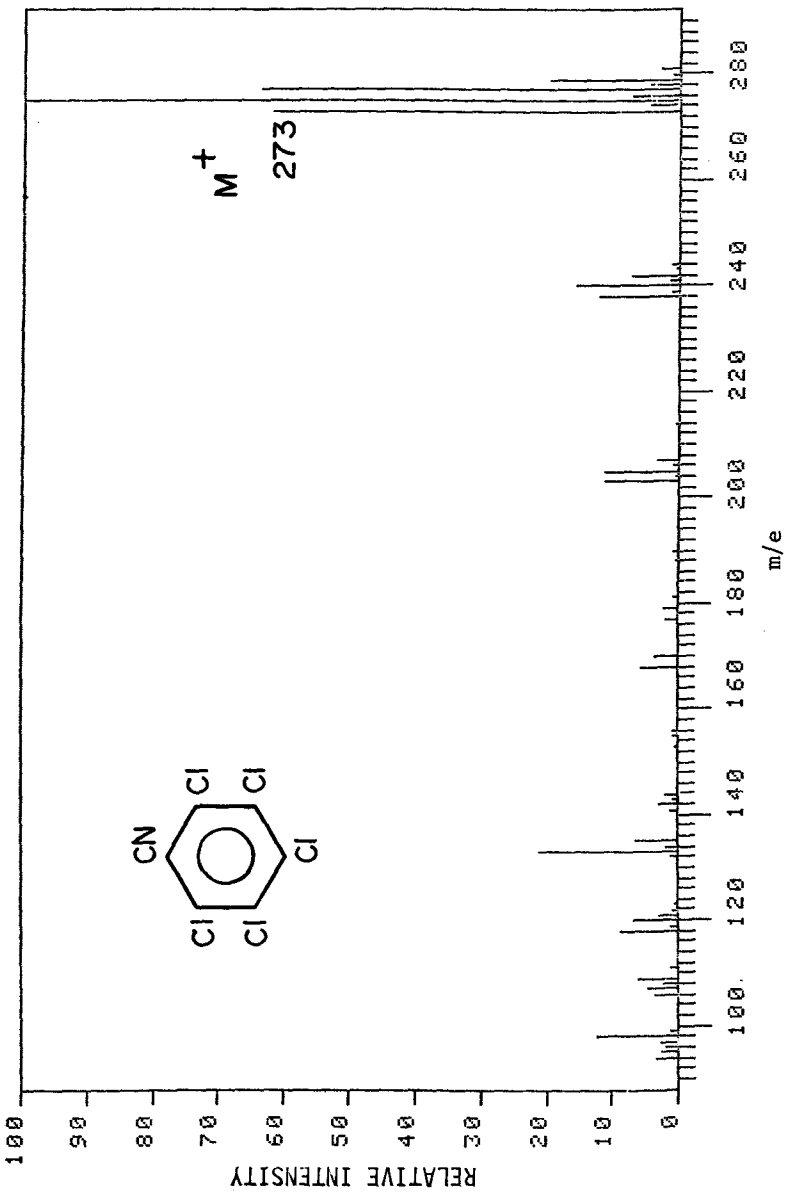


Fig. 1. Mass Spectrum of Pentachlorobenzonitrile

TABLE 1

Accurate Mass Measurement and Elemental Composition Data for Pentachlorobenzonitrile

| $M^+$ Isotope Cluster | | Measured Mass | Calculated Mass |
|---|---|---|---|
| $M^+$ | $C_7N^{35}Cl_5$ | 272.8479 | 272.8474 |
| $M^+$+2 | $C_7N^{35}Cl_4{}^{37}Cl_1$ | 274.8439 | 274.8434 |
| $M^+$+4 | $C_7N^{35}Cl_3{}^{37}Cl_2$ | 276.8415 | 276.8415 |
| $M^+$+6 | $C_7N^{35}Cl_2{}^{37}Cl_3$ | 278.8397 | 278.8384 |
| ($M^+$-Cl) Isotope Cluster | | | |
| $M^+$-Cl | $C_7N^{35}Cl_4$ | 237.8772 | 237.8785 |
| ($M^+$-Cl)+2 | $C_7N^{35}Cl_3{}^{37}Cl_1$ | 239.8748 | 239.8755 |
| ($M^+$-Cl)+4 | $C_7N^{35}Cl_2{}^{37}Cl_2$ | 241.8737 | 241.8726 |
| ($M^+$-2Cl) Isotope Cluster | | | |
| $M^+$-2Cl | $C_7N^{35}Cl_3$ | 202.9175 | 202.9096 |
| ($M^+$-2Cl)+2 | $C_7N^{35}Cl_2{}^{37}Cl_1$ | 204.9027 | 204.9031 |
| ($M^+$-2Cl)+4 | $C_7N^{35}Cl_1{}^{37}Cl_2$ | 206.8957 | 206.9037 |
| ($M^+$-3Cl) Isotope Cluster | | | |
| $M^+$-3Cl | $C_7N^{35}Cl_2$ | 167.9394 | 167.9408 |
| ($M^+$-3Cl)+2 | $C_7N^{35}Cl_1{}^{37}Cl_1$ | 169.9350 | 169.9378 |
| ($M^+$-4Cl) Isotope Cluster | | | |
| $M^+$-4Cl | $C_7N^{35}Cl$ | 132.9709 | 132.9720 |
| ($M^+$-4Cl)+2 | $C_7N^{37}Cl$ | 134.9633 | 134.9689 |
| $M^+$-5Cl | $C_7N$ | 98.0019 | 98.0031 |

cide chlorthalonil (tetrachloroisophthalonitrile; $C_6Cl_4(CN)_2$) and may be present in quantities of up to 2% (GLASGOW, 1976). Chlorthalonil was not detected in either the 6% or 15% ether-petroleum ether Florisil fractions collected from the carrot samples. However, several celery samples subsequently collected from a different grower in the same geographic region exhibited residues of chlorthalonil in the 50% ether-petroleum ether fraction in addition to pentachlorobenzonitrile in the 15% ether-petroleum ether fraction.

Relative retention times (aldrin = 1.00) for pentachlorobenzonitrile were 0.5 and 0.66 on columns A and B, respectively and the material appeared to be approximately twice as sensitive as heptachlor epoxide under these conditions.

Preliminary recovery studies indicate (Table 2) that pentachlorobenzonitrile can be quantitatively recovered by the official procedure (AOAC, par. 29.011, 1975) for non-fatty foods and partially recovered by the fatty food procedure (AOAC, par. 29.012, 1975). Elution from the Florisil column with the normal amount (200 ml) of 15% ether-petroleum ether is incomplete for butter but is improved by elution with an additional 200 ml. of eluent.

TABLE 2

Recovery Data for Pentachlorobenzonitrile In Some Foods

| Food | Method | Spiking Level (ppm) | Percent Recoveries |
|---|---|---|---|
| Peas | non-fatty | 0.01 | 102.4, 106.4 |
| Yams | non-fatty | 0.01 | 89.6, 91.1 |
| Butter | fatty | 0.30 | 57.1, 65.7 |
| Butter | fatty[a] | 0.3 | 74.3, 68.6 |

[a] Elution volume of 15% ethyl ether/petroleum ether increased to 400 ml.

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