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Occurrence of Pentachloronitrobenzene and its Metabolites in Spinach Leaves

Thomas Cairns, Emil G. Siegmund, and Herbert T. Masumoto

Department of Health and Human Services, Food and Drug Administration, 1521 West Pico Boulevard, Los Angeles, CA 90015

The primary use of pentachloronitrobenzene (PCNB) (1) as a soil fungicide to control Rhizoctonia in cotton (Gossypium hirsutum) and Sclerotium in peanut (Arachis hypogaea) has been widespread for some time (EPA. 1976). Use as a foliar fungicide on certain vegetable crops has also been prevalent. Extensive metabolic studies of PCNB in the peanut have been reported by LAMOUREUX and RUSNESS (1980). Three competing reactions were suggested for PCNB metabolism: aryl nitro reduction, nucleophilic displacement of a nitro group, and nucleophilic displacement of a chloro group. In an additional study (RUSNESS & LAMOUREUX, 1980) of the chloroform soluble metabolites produced in vivo (hydroponically grown peanuts) by PCNB, the major product via aryl nitro reduction was identified as pentachloroaniline (PCA) (2) with pentachlorothioanisole (PCTA) (3) also being identified as a

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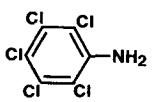

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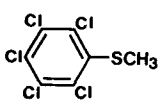

(3)

minor product through catabolic reaction via S-(pentachlorophenyl)cysteine. On the issue of translocation of PCNB and its metabolites from the roots to the foliar tissue, these authors concluded that such processes were very restricted (about 3% on the average). In a study of PCNB translocation in the bean (Phaseolus vulgaris), BRISTOW et al. (1973) concluded that no PCNB could be detected in the leaves while it was determined to be in the cotyledons.

While residues of PCNB, PCA, and PCTA in below ground plants grown in PCNB treated soil are quite common (HEIKES, 1980), it has been unclear as to the extent and incidence of PCNB and its metabolites in the foliar tissue of edible legumes. This paper describes the identification procedure for PCNB and its metabolites by methane chemical ionization (CI) mass spectrometry in spinach leaves. The quantitation of PCNB by GCMS was performed at the ppm level. Speculation and arguments are advanced as to the origin of the residue levels found.

MATERIALS AND METHODS

Mass Spectral Data. All spectra were obtained on a Finnigan Model 3300 quadrupole mass spectrometer equipped with a CI source and INCOS Data System; operating conditions: 90 cm x 2 mm i.d. glass column packed with 2% DEGS on 80/100 mesh Chromosorb W; carrier gas and reagent gas, 30 mL methane/min; column inlet, 250°C; column temperature, 180°C, isothermal.

Sample Preparation. A 100 g portion of the sample was extracted by sec 212.13a of the PESTICIDE ANALYTICAL MANUAL (VOLUME 1, 1982), and eluted through Florisil using the 6% eluate of sec. 211.14d of the PESTICIDE ANALYTICAL MANUAL (Volume 1, 1982) and then concentrated to 0.1 mL for direct injection into the GCMS system.

RESULTS AND DISCUSSION

Preliminary analysis of the spinach extract by gas chromatography equipped with a Hall electrolytic conductivity detector (HECD) (LUKE et al. 1975, 1981) revealed the presence of three chlorinated compounds. From extensive correlation tables on retention data of pesticides and industrial chemicals, the major response was thought to be PCNB. The case was referred to gas chromatography mass spectrometry (GCMS) for confirmation and structural identity of the remaining two unidentified analytical responses (UARs). Since PCNB was suspected to be present, the choice of chemical ionization (CI) GCMS was made to rely heavily on the production of protonated molecular ions and hence providing a convenient method of confirmation. Previous analysis by electron impact MS (HEIKES 1980) had produced a complex fragmentation with certain features shared by its metabolites. Additionally, the presence of two UARs in the sample could possibly be attributed to the known metabolites of PCNB (i.e. PCA and PCTA). Using the GCMS-CI approach, the spectra of PCNB (**1**), PCA (**2**) and PCTA (**3**) were obtained (Fig. 1). The dominant spectral feature of three spectra was the singular appearance of protonated molecular ion clusters. Such clusters could provide a solid base for facile identification as illustrated in Figure 2. Clearly, the presence of a five chlorine containing entity was established. By employing a 2% DEGS column the retention time of PCNB and its metabolites were sufficiently separated. Selecting the mass ranges expected for the three molecular weights, m/z 294-299 for PCNB, m/z 299-300 for PCTA (to avoid mass range overlap with PCNB), and m/z 264-269 for PCA, mass chromatograms from the total ion current can be considered definitive for confirmation purposes. The spinach extract was then examined under exactly similar conditions and the results are displayed in Figure 3. Clearly the presence of PCNB and its two metabolites have been established in the sample. Quantitation of the PCNB level found

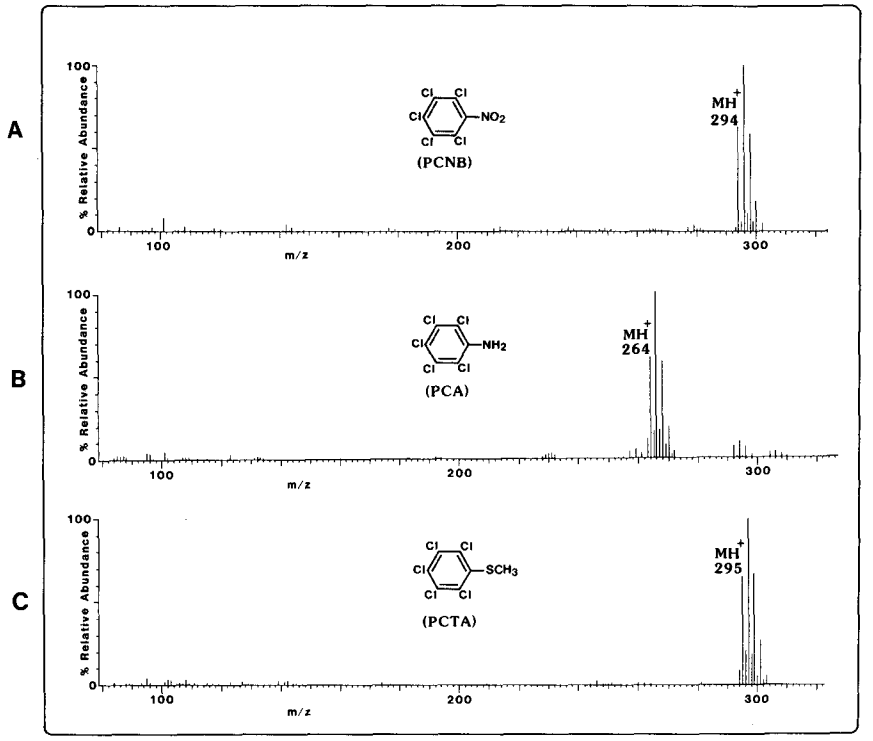

Figure 1. Methane chemical ionization mass spectra of (A) pentachloronitrobenzene (PCNB), (B) pentachloroaniline, and (C) pentachlorothioanisole (PCTA) recorded under GCMS conditions.

was accomplished by comparision of area measurements for the single ion monitoring of m/z 296. A standard injection of PCNB was adjusted until its recorded area matched the sample response. The resultant level found by this technique was 0.13 ppm which agreed well with the value of 0.14 ppm calculated by HECD.

The foliar occurrence of the PCNB and its metabolites PCA and PCTA deserves speculative comment. Previous studies by LAMOUREUX and RUSNESS (1981) have already declared that translocation from the roots to the foliar tissue (in peanuts) is very restrictive. It could be argued that the levels found are from (a) a restrictive translocation from a soil very high in PCNB and its metabolites, (b) direct contact contamination from the soil itself, and (c) foliar metabolism after aeriel spraying of PCNB directly onto the spinach leaves. While the most studied solution seems to be translocation, it is difficult to visualize the necessary high concentrations of PCNB in the soil at crop

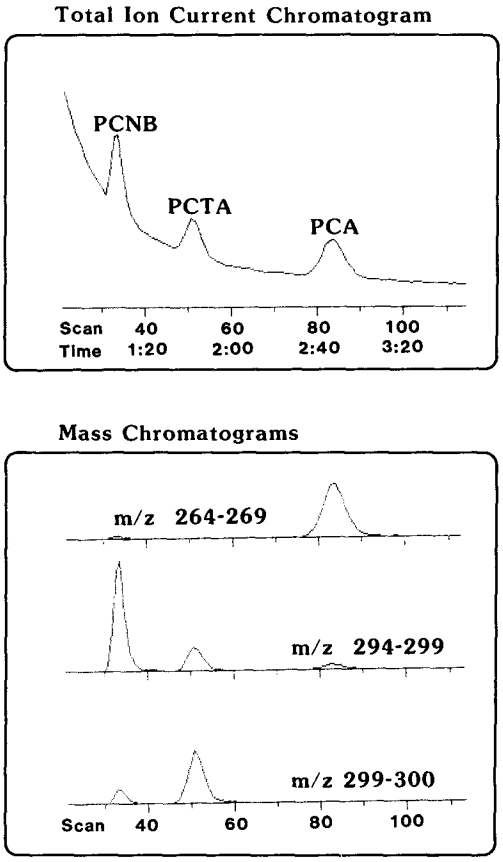

Figure 2. Total ion current chromatogram of a standard mixture of PCNB, PCA and PCTA with three mass chromatograms: m/z 294-299 for PCNB, m/z 299-300 for PCTA and m/z 264-269 for PCA.

harvesting time to account for the 0.13 ppm level found in the spinach leaves. This mechanism must be considered even less likely since the model study was a hydroponic one and the normal agricultural situation should result in a more restrictive translocation. On the other hand, contact transmission from the soil does seem highly improbable. The last option of foliar metabolism might well be the operative mechanism to explain the results obtained. However, the data base for such a conclusion is insufficient. Perhaps further incidences of PCNB and its metabolites in spinach leaves and other legumes would assist in establishing such residues as a result of direct crop spraying rather than the major proper use as a soil funcigide.

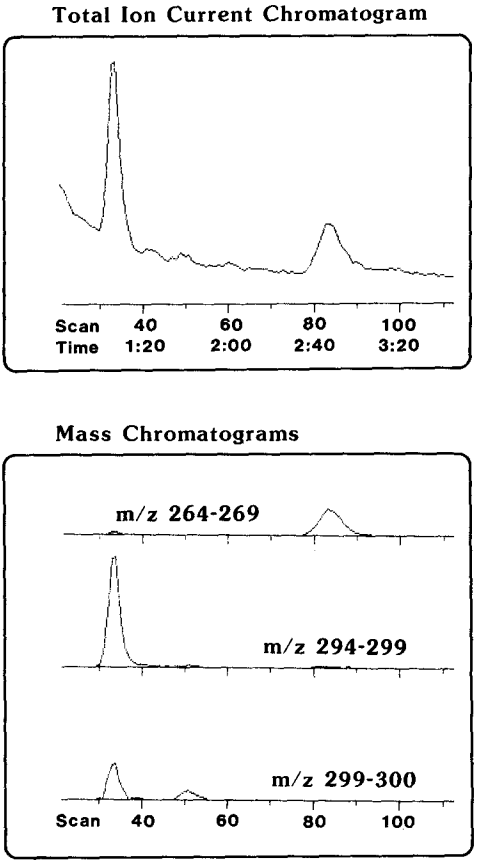

Figure 3. Total ion current chromatogram of spinach extract with resultant mass chromatograms to detect PCNB, PCA and PCTA.

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