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Residues of Pentachloronitrobenzene and Related Compounds in Peanut Butter

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Pentachloronitrobenzene (PCNB, Quintozene^{R}, Terraclor^{R}) is used as a soil fungicide and seed disinfectant on peanuts. The recommended application rate is 5-200 lb actual/acre or 1/2 - 3/4 lb actual/bushel seed (THOMPSON 1978).

Residues of PCNB have been found during the examination (by gas chromatography) of several peanut butter samples from the Total Diet Program conducted by the Food and Drug Administration (MANSKE and JOHNSON 1977). These chromatograms often contain several other peaks thought to be related to PCNB. The identity of these compounds has been determined by gas-liquid chromatography/mass spectrometry (GC/MS).

EXPERIMENTAL

Routinely, peanut butter from each market basket is analyzed as part of a composite of fats and oils (i.e., margarine, shortening, and salad dressing). In this study, however, 11 peanut butter samples were examined individually by methods currently used by the Food and Drug Administration for multiresidue analysis:

(a) The samples were hydrolyzed by heating with 10% H_2SO_4 at 100^{o} for one hour in a closed tube.

(b) The fats and oils were extracted from the samples with a mixture of ethyl ether and petroleum ether (AOAC, par 29.012(d), 1975).

(c) Removal of the fats and oils was accomplished with a gel permeation column of OR PVA 2000 beads eluted with 30% CH_2Cl_2 in acetone (HOPPER 1978). One half of the resulting eluate was methylated with diazomethane.

(d) The methylated and unmethylated portions of the eluate were each passed through a Florisil column (MILLS et al. 1972). Eluant A (20% CH_2Cl_2-hexane) was analyzed by gas chromatography with electron capture detection (EC/GC).

EC/GC: A Tracor 560 gas chromatograph with a ^{63}Ni detector and linearizer was fitted with an inlet splitter (1:6) and used for quantitative analysis. Column parameters were as follows: Glass capillary, 30 m x 0.5 mm (ID), OV-101 wall coated open tubular (WCOT) column at 140^{o}, with helium carrier gas at 6 ml/min flow.

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GC/MS: A DuPont 491B mass spectrometer was interfaced through a glass jet separator to a Varian 2740 gas chromatograph. Samples were injected on a 1.8 m x 2 mm pyrex column packed with 3% OV-1 on Chromosorb WHP 60/80 mesh at 180° and 30 ml/min He flow. The injector temperature was 220°.

The data system was comprised of a Hewlett-Packard 2100A computer (16K), and 7900A disc drive, with Tektronix 4010 teletype port interface and 4610 hard copy unit.

Electron impact spectra (75 eV) were obtained at a scan rate of 2 sec/dec, scanning from 517 to 41 amu. The source was held at 240° with an accelerating voltage of 1.4 KV.

RESULTS AND DISCUSSION

The compounds found in peanut butter are listed in Table 1. Their retention data, relative to pentachlorobenzene (QCB) is included.

TABLE 1
Compounds Found in Peanut Butter

Identity	Relative Retention (QCB=1.00; 1.34 min) OV-101, WCOT, 140°
Pentachlorobenzene (QCB)	1.00
Tetrachloronitrobenzene (TCNB)[1]	1.48
Hexachlorobenzene (HCB)	2.31
Pentachloroanisole (PCAS)[2]	2.49
Pentachloronitrobenzene (PCNB)	3.01
Pentachloroaniline (PCA)	5.75
Pentachlorothioanisole (PCTA)	6.58
Pentachlorophenol (PCP)[3]	

[1] Relative retention identical to 2,3,5,6-tetrachloronitrobenzene.
[2] This abbreviation is used to avoid confusion with pentachloroaniline.
[3] Determined as the methyl ether-pentachloroanisole.

An assay of Terraclor® was performed. The commercial product was simply diluted with ethyl acetate to a concentration compatible with electron capture sensitivity. The results of this assay are given in Table 2.

The results of analysis of eleven peanut butter samples are listed in Table 3. All quantitation was performed using capillary column EC/GC. Figure 1 represents a typical chromatogram. Identification or confirmation was accomplished with GC/MS.

TABLE 2
Assay of Terraclor®

Identity	%
Pentachloronitrobenzene (PCNB)	99.2
Hexachlorobenzene (HCB)	0.46
Pentachlorobenzene (QCB)	0.17
Tetrachloronitrobenzene (TCNB)[1]	0.06

[1] Calculated as 2,3,5,6-tetrachloronitrobenzene.

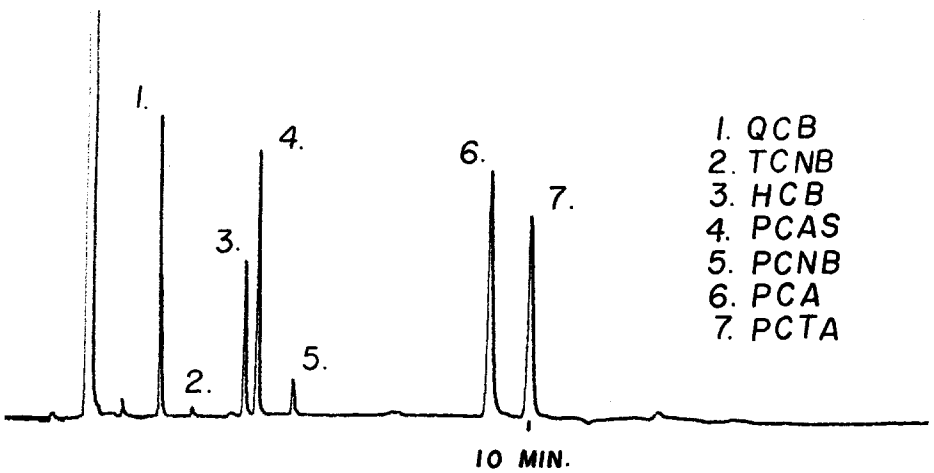

Figure 1 - A typical chromatogram of a peanut butter sample (OV-101 WCOT glass column, 30 m x 0.5 mm, 140°, ^{63}Ni).

Residues of QCB, TCNB, HCB, pentachloroanisole (PCAS), PCNB, PCA, PCTA and PCP were found in each of the eleven peanut butter samples examined. The concentrations of PCP were calculated from the differences in the levels of PCAS in the methylated and unmethylated eluates. All eight compounds showed good recovery (>85%) through this procedure.

All eleven samples were also analyzed by a procedure identical to that listed above, except that the hydrolysis step was eliminated. The values of PCP averaged 52% less without hydrolysis, but the other values showed no change.

TABLE 3

Results of analysis of eleven peanut butter samples.
All values are expressed as ppb.

Identity	Sample											
	1	2	3	4	5	6	7	8	9	10	11	Ave.
Pentachlorobenzene (QCB)	1.8	4.4	5.0	7.7	6.9	5.5	11	17	23	29	62	16
Tetrachloronitrobenzene (TCNB)[1]	0.63	0.60	0.91	0.42	0.73	1.0	0.62	1.6	1.3	4.3	0.31	1.1
Hexachlorobenzene (HCB)	0.97	1.7	2.0	2.2	3.1	3.8	5.1	5.3	5.6	14	38	7.4
Pentachloroanisole (PCAS)[2]	1.5	3.3	3.5	9.1	5.8	3.6	8.7	9.9	11	17	33	9.7
Pentachloronitrobenzene (PCNB)	0.80	2.5	3.1	3.5	5.5	2.8	6.8	10	6.3	9.4	2.9	4.9
Pentachloroaniline (PCA)	3.4	16	21	13	17	11	40	63	34	100	140	42
Pentachlorothioanisole (PCTA)	2.6	9.9	18	2.4	9.5	7.7	35	40	27	42	61	23
Pentachlorophenol (PCP)	64	18	21	18	17	36	26	12	27	21	53	28

[1] Calculated as 2,3,5,6-tetrachloronitrobenzene.
[2] This abbreviation is used to avoid confusion with pentachloroaniline.

PCNB is fairly rapidly converted to pentachloroaniline (PCA) by various soil microorganisms. Although PCA is less inhibitory to soil fungi, it is much more stable than PCNB (KO and FARLEY 1969). The metabolism of PCNB to pentachlorothioanisole (PCTA) in soil has been proven by a number of investigators (DEJONCKHEERE et al. 1975 and IWATA et al. 1978). However, while the metabolism of PCNB to PCA is common to various microorganisms, the metabolism of PCNB to PCTA is specific for only a few filimentous fungi (NAKANISHI and OKU 1969).

Hexachlorobenzene (HCB) and pentachlorobenzene (QCB) have been reported at significant levels in both soil treated with PCNB and in crops grown on these soils. These two persistent compounds are impurities in PCNB formulations and accumulate in treated soil (SMELT and LEISTRA 1974 and HAEFNER 1975). The level of QCB was found to be higher than that of HCB in all eleven samples examined. This was not expected since HCB was found at higher concentrations in the Terraclor® sample (see Table 2). It has, however, been shown that PCNB may be reduced to QCB by UV light (CROSBY and HAMADMAD 1971). This photochemical reaction may account for the higher than anticipated levels of QCB. The fairly wide use of TCNB as a fungicide and growth regulator may account for its significant residue levels in these samples.

PCP and PCAS have not been previously reported as impurities or metabolites of PCNB. However, their consistent presence in these samples leads to speculation that these chemicals may be related to PCNB. A statistical correlation using environmental levels of PCNB is probably futile since it is quite rapidly metabolized. However, environmental levels of HCB should reflect initial levels of PCNB since it is a stable impurity. This theory is supported by correlation coefficients of 0.92 for HCB vs. PCA and 0.81 for HCB vs. PCTA. PCA and PCTA are known metabolites of PCNB. A correlation coefficient of 0.96 was found for HCB vs. PCAS, strongly suggesting that PCAS is a metabolite of PCNB.

Essentially no correlation was found between the levels of HCB and PCP; probably because PCP is nearly ubiquitous in the environment. Additionally, sample number 1 (see Table 3) was found to be contaminated by PCP. The paper liner in the jar lid of this sample was found to contain nearly 40 micrograms of PCP. The sample was unemulsified and the oily upper layer contacted the paper liner.

Since it is well known that PCP is metabolized to PCAS in soil (KUWATSUKA and IGARASHI 1975), it is logical to assume that PCNB is first metabolized to PCP which, in turn, is metabolized to PCAS. Soil studies are being conducted to test this hypothesis.

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