Formation of Methyl Pentachlorophenyl Sulfoxide and Sulfone from Pentachloronitrobenzene in Soil and Plants

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Pentachlorothioanisole (PCTA), a metabolite of pentachloronitrobenzene (PCNB), was converted to methyl pentachlorophenyl sulfoxide (PCTA-SO) and methyl pentachlorophenyl sulfone (PCTA-SO₂) in soil under laboratory conditions. The conversion was hardly observed in autoclaved soil, suggesting participation of microorganisms in the process. The presence of PCTA-SO and PCTA-SO₂ was also confirmed in soil from a field plot treated with PCNB and in peanuts harvested from the plot. A trace amount of PCTA-SO was also detected in young corn plants grown in a culture solution containing PCNB.

INTRODUCTION

Pentachloronitrobenzene (PCNB) is widely used as a fungicide to control clubroot of cruciferous vegetables and root rot of sugar beet in Japan.

Metabolism and degradation of PCNB have been investigated in mammals,¹² plants,²,³ and soils.⁴⁻⁹ The principal metabolites, pentachloroaniline (PCA) and pentachlorothioanisole (PCTA), have been identified in both microbial systems⁶,¹⁰ and soils.⁷⁻⁹ Recently, pentachlorophenol was also detected in soil as a degradation product.⁹

It has been shown that pesticides with the thioether group, such as demeton,¹¹ fenthion,¹² carboxin,¹³ Denmert®,¹⁴,¹⁵ and thio-carbamate herbicides,¹⁶ are frequently metabolized to corresponding sulfoxides and sulfoines in mammals, plants, and soils. It was, therefore, expected that PCTA derived from PCNB would also be metabolized to the corresponding sulfoxide and sulfone. These compounds, however, have not yet been found.

The purpose of this investigation is to examine the production or presence of the sulfoxide and sulfone in soil, plants, and microorganisms.

A preliminary report¹⁷ has already been presented.

MATERIALS AND METHODS

1. Chemicals

Methyl pentachlorophenyl sulfoxide (PCTA-SO) and methyl pentachlorophenyl sulfone (PCTA-SO₂) were prepared according to the procedure used for the preparation of thiocarbamate sulfoxides and sulfoines.¹⁶

Methyl pentachlorophenyl sulfoxide. A solution of m-chloroperbenzoic acid (5 mmole) in chloroform (25 ml) was added gradually with stirring to PCTA (5 mmole) in chloroform (25 ml) at room temperature and the mixture was held for 1 hr at the same temperature. The reaction mixture was washed twice with 0.1 N NaOH and then with water, and dried over anhydrous sodium sulfate. The solvent was evaporated and the residue was subjected to preparative tlc with a 2:1:1 mixture of hexane, benzene and ether. The PCTA-SO separated by tlc was crystallized from 20% (v/v) benzene–hexane to give colorless plates, mp 161.5°C (Found: C, 27.05; H, 0.99; Cl, 56.92; S, 10.45; C₇H₃Cl₅O₃S requires C, 26.91; H, 0.97; Cl, 56.74; S, 10.26%).

Methyl pentachlorophenyl sulfone. A solution of m-chloroperbenzoic acid (15 mmole) in chloroform (25 ml) was added gradually with stirring to PCTA (5 mmole) in chloroform (25 ml) at room temperature and the mixture...
was allowed to stand overnight. The reaction mixture was then purified in a similar manner to that of PCTA-SO. Recrystallization from 20% (v/v) benzene-hexane gave colorless fine needles, mp 188-189°C (Found: C, 25.73; H, 0.93; Cl, 54.00; S, 9.72; C12H11Cl5O2S requires C, 25.60; H, 0.92; Cl, 53.97; S, 9.76%).

In GC-MS analysis, the sulfoxide and the sulfone gave clusters of molecular ion peaks at and above m/e 310 and m/e 326, respectively, which are in agreement with calculated values for PCTA-SO and PCTA-SO2.

2. Treatments

A top soil from the field of National Institute of Agricultural Sciences, Nishigahara, Tokyo was used for the laboratory experiment. The soil was passed through a 2-mm sieve prior to use. The physicochemical properties of the soil were as follows: volcanic ash soil; clay loam, clay content 29.0%, total carbon 4.1%, pH 6.9, C.E.C. 33.1 meq/100 g dry soil, maximum water-holding capacity 89.7%. Seventy grams of the soil (on an oven-dried weight basis) was put into each of 200-ml Erlenmeyer flasks and treated with 2 mg of PCNB or PCTA in 1 ml of acetone. The soil was moistened to 50% of the maximum water-holding capacity and incubated at 28°C for a month. The soil taken in two other flasks was autoclaved at 121°C, treated with PCTA aseptically and incubated at the same moisture condition. The untreated soil was used as control. Each treatment was carried out in duplicate. The moisture content of the soil was adjusted by the addition of water after 15 days.

Soil samples from experimental plots at Ibaraki Agricultural Experiment Station in Mito were also analyzed for these compounds. The surface 10-cm soil of the plots had been mixed with PCB dust at the rate of 80 kg (on the basis of active ingredient) per hectare for the control of clubroot of Chinese cabbage (Brassica pekinensis R. cv. Ohsho) in September 1973 before planting the seedlings. Peanut plants (Arachis hypogaea L. cv. Chibahanryu) were grown on the plots from May to October, 1974. The harvested peanuts were air-dried well, separated from the shells and stored at room temperature for 40 months until analysis. The soil was collected from the plots in June, 1974 and stored at 10°C for 33 months until analysis. The physicochemical properties of the soil were as follows: volcanic ash soil; clay loam, clay content 16.2%, total carbon 7.1%, pH 5.0, C.E.C. 23.3 meq/100 g dry soil, maximum water-holding capacity 66.3%. Control plot was located adjacent to the treated plot.

Seeds of corn (Zea mays L. cv. Golden cross bandam) were germinated and allowed to grow in vermiculite which was intermittently sub-irrigated with a half-strength Hoagland’s solution. The young plants were removed from the vermiculite at the four-leaf stage and the roots were washed. Each of ten plants was inserted in a 200-ml Erlenmeyer flasks containing 100 ml of the half-strength Hoagland’s solution in which PCNB was added at 2 ppm with one drop of Tween-80. The flasks were placed in the greenhouse at 25±3°C. The initial level of the culture solution was maintained by adding the half-strength Hoagland’s solution periodically. After 10 day’s incubation, the plants were harvested and washed with water and then with methanol to remove the surface residues. The shoots of the plants were subjected to analysis.

3. Extraction and Purification

Soil: One hundred ml of 80% acetone was added to 30 g of soil sample and the mixture was occasionally shaken for 2 hr by hand and allowed to settle. Twenty-five ml portion of the supernatant solution was transferred into a separatory funnel with 75 ml of 20% NaCl solution. The contents were mixed and then extracted twice each with 50 ml of benzene. The combined benzene extracts were washed with 50 ml of 0.1 N KOH and with 50 ml of 20% NaCl, dried over anhydrous sodium sulfate and then concentrated to near dryness on a rotary evaporator at 45-50°C. The residue was dissolved in 5 to 10 ml of benzene and cleaned up by Florisil column chromatography.

Corn plant: The shoots of corn plants (32 g) were chopped into pieces of about 1 cm long and homogenized in a Waring Blender with 60 ml of acetone. The homogenate was filtered through a sintered-glass filter (No. 3). The residue was washed twice each with 30 ml of acetone. The filtrate and washings were
combined and transferred into a separatory funnel and 260 ml of 20% NaCl was added. The contents were mixed and then extracted twice each with 50 ml of benzene. The benzene extracts were combined, washed with 50 ml of 0.1 N KOH and with 50 ml of 20% NaCl, dried over anhydrous sodium sulfate, and subjected to Florisil column chromatography.

Peanut: Air-dried peanuts were ground to fine powder with a mortar and pestle. Ten grams of the powder was taken into a 200-ml Erlenmeyer flask and 50 ml of an acetone-hexane (1:2) mixture was added to the flask. The contents were occasionally shaken for 2 hr by hand and then filtered through a sintered-glass filter (No. 3). After washing twice each with 20 ml of the solvent mixture, the residue with large particles was transferred into the mortar and ground with 30 ml of the mixture. The ground residue was filtered and remaining solids were washed with a small volume of the mixture. The filtrate and washings were combined and concentrated to near dryness on a rotary evaporator at 45-50°C. The residue was subjected to hexane-acetonitrile partitioning and then cleaned up by Florisil column chromatography.

4. Chromatography
Florisil column chromatography was conducted as follows: Five grams of Florisil (60-80 mesh) containing 5% of water was placed in a chromatographic column of 10 mm in i.d. by the wet method with hexane, and topped with 5 g of anhydrous sodium sulfate. The extracts of samples were transferred onto the column using a small volume of benzene. PCNB, PCTA and PCA were eluted with 100 ml of 6% ether in hexane and PCTA-SO and PCTA-SO₂ subsequently with 100 ml of 15% ether in benzene. The eluates were evaporated to near dryness and the residue was quantitatively transferred into 10-ml volumetric flasks.

Thin-layer chromatography (tle) was conducted as follows: Silica gel 60F-254 plates (E. Merck) of 0.25 mm thickness and 0.5 mm thickness were used for analytical and preparative purposes, respectively. The Rf values of tle for the reference compounds on the plates and solvent systems used are given in Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rf in solvent system</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>PCNB</td>
<td>0.70</td>
</tr>
<tr>
<td>PCTA</td>
<td>0.81</td>
</tr>
<tr>
<td>PCA</td>
<td>0.42</td>
</tr>
<tr>
<td>PCTA-SO</td>
<td>0</td>
</tr>
<tr>
<td>PCTA-SO₂</td>
<td>0</td>
</tr>
</tbody>
</table>

Solvent system: A, cyclohexane; B, hexane-benzene-ether (2:1:1). Stationary phase: silica gel 60F-254, 0.25 mm in thickness.

Gas-liquid chromatography (glc) was conducted with a Shimadzu model GC-5A equipped with an electron-capture detector (63Ni, 10 mCi). The operating conditions are as follows: A pyrex column (3 mm in i.d. and 2 m in length) was packed with 1% silicone XE-60 on 60-80 mesh Chromosorb W (AW-DMCS). The flow rate of carrier gas (N₂) was 100 ml per minute. The column temperature was 180°C for PCNB, PCTA and PCA, and 250°C for PCTA-SO and PCTA-SO₂. Under these conditions, retention times of PCTA-SO and PCTA-SO₂ were 1.7 and 2.4 min, respectively.

Recoveries of PCTA-SO and PCTA-SO₂ were 70-80% or higher in all the experiments. Identification of PCTA-SO and PCTA-SO₂ was accomplished by a combination of Florisil column-chromatography, tle and glc.

RESULTS AND DISCUSSION

Two unknown metabolites with the same retention times as those of PCTA-SO and PCTA-SO₂ were detected in PCNB-treated soil. To confirm these metabolites as PCTA-SO and PCTA-SO₂, the extracts were purified by tle. The fraction of 15% ether-benzene from Florisil column was subjected to tle with cyclohexane. The zone of origin where PCTA-SO and PCTA-SO₂ were located on the plate was scraped off from the plate and extracted with a 1:1 mixture of acetone and benzene. The extracts were concentrated to near dryness under reduced pressure. The residues were
then developed on a tlc plate with a 2:1:1 mixture of hexane, benzene and ether. Authentic PCTA-SO and PCTA-SO₂ were spotted and developed on the same plate. Each zone corresponding to PCTA-SO or PCTA-SO₂ was scraped off from the plate, and then extracted with a mixture of acetone and benzene. These extracts were concentrated and then subjected to glc. Retention times of the metabolites from the extracts coincided with those of authentic PCTA-SO and PCTA-SO₂. Thus, the behavior of these two metabolites were quite identical with those of authentic PCTA-SO and PCTA-SO₂. On the basis of these results, it was concluded that these two metabolites were PCTA-SO and PCTA-SO₂.

Table 2 shows the concentrations of PCTA-SO and PCTA-SO₂ in the soils which were treated with PCNB or PCTA and incubated for a month. Levels of PCTA-SO and PCTA-SO₂ in the PCTA-treated soil were 20 to 30 times as high as those in the PCNB-treated soil. It was, therefore, supposed that PCTA derived from PCNB was further converted to the sulfoxide and sulfone in the soil. The level of PCTA-SO in an autoclaved soil was only one-hundredth of that in an unsterilized soil and PCTA-SO₂ could not be detected in the autoclaved soil. These results suggest the participation of soil microorganisms in the production of PCTA-SO and PCTA-SO₂ in the soil. Although a small amount of PCTA-SO was produced in the autoclaved soil, no further check was made to determine whether the production was due to nonbiological oxidation or contamination of microorganisms. PCTA-SO and PCTA-SO₂ were also found at the levels of 0.48 and 0.21 ppm, respectively, in

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration treated*, ppm</th>
<th>Concentration found*, (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCNB</td>
<td>PCTA</td>
</tr>
<tr>
<td>Soil</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soil + PCNB</td>
<td>28.5</td>
<td>12.3</td>
</tr>
<tr>
<td>Autoclaved Soil + PCTA</td>
<td>28.5</td>
<td>19.4</td>
</tr>
<tr>
<td>Soil + PCTA</td>
<td>28.5</td>
<td>13.6</td>
</tr>
</tbody>
</table>

* Oven-dried basis. b) Average of two replicates. e) Limit of detection is 0.005 ppm.

Table 3 Residues of PCNB and its metabolites in soils from field treated with PCNB.

<table>
<thead>
<tr>
<th>PNCB application rate (kg/ha)</th>
<th>Residue*, (ppm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>PCNB</td>
</tr>
<tr>
<td>0</td>
<td>0.32</td>
</tr>
<tr>
<td>80</td>
<td>14.1</td>
</tr>
</tbody>
</table>

* Oven-dried basis. b) Average of two replicates. e) Limit of detection is 0.005 ppm.

Table 4 Residues of PCNB and its metabolites in peanuts harvested from the field plots treated with PCNB.

<table>
<thead>
<tr>
<th>PNCB application rate (kg/ha)</th>
<th>Residue*, (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCNB</td>
</tr>
<tr>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>80</td>
<td>0.11</td>
</tr>
</tbody>
</table>

* Average of two replicates. b) Air-dried weight basis. e) Limit of detection is 0.002 ppm.
the soil from the field plot treated with PCNB (Table 3). Low levels of these metabolites were found in the control soil, which may be attributable to the contamination in the field plots.

These metabolites were also found in peanuts from the PCNB-treated field plot. Table 4 shows the residue levels of PCNB and its metabolites in peanuts. The levels of PCTA-SO and PCTA-SO$_2$ were 0.006 and 0.002 ppm, respectively.

In corn plants, PCTA-SO was also present at 0.007 ppm, but PCTA-SO$_2$ could not be detected (Table 5).

The conversion of PCNB to PCTA-SO and PCTA-SO$_2$ in microorganisms was investigated using *Fusarium oxysporum* f. *lycopersici* which is known to metabolize PCNB to PCTA.$^{10}$ The fungus was incubated in Cazapek Dox medium containing 10 ppm of PCNB. PCTA, PCA and PCTA-SO were contained in the culture broth at 0.09, 0.3 and 0.00007 ppm, respectively, but PCTA-SO$_2$ could not be detected.

A variety of sulfur compounds are frequently metabolized to sulf oxides and sulfones. Although the conversion of PCNB to PCTA has been established and the possibility of further oxidation of PCTA to PCTA-SO and PCTA-SO$_2$ was expected, the presence of these metabolites have not hitherto been reported. This may be attributed to their presence in extremely small quantities and to their long retention times on gie under conditions suitable for PCNB.

In the present study, about 5% of total PCTA added to the soil was converted to PCTA-SO and PCTA-SO$_2$, respectively, when the soil was incubated under aerobic conditions. On the other hand, Murthy *et al.* presumed that PCA and PCTA are end products in the degradation of PCNB in an anaerobic soil.$^{9}$

Igarashi *et al.* analyzed both the $^{14}$C–PCNB-treated soil and shoots of corn plants incubated in culture solution containing $^{14}$C–PCNB.$^{3,7}$ They observed that 15–20% of the total radioactivity from soil and 88% of the total radioactivity from the shoots were located on the origin on TLC plates developed with cyclohexane. They designated the radioactive substance(s) on the origin as unknown I (UK$_1$), but no further analysis was conducted. PCTA-SO and PCTA-SO$_2$ might be contained in the fraction of UK$_1$ since they behaved like UK$_1$ on the TLC plate when developed with cyclohexane.

Based on the results obtained in this investigation, PCTA derived from PCNB seems to be readily metabolized, like a variety of sulfur compounds, to its sulfoxide and sulfone in the environment.

**ACKNOWLEDGEMENT**

The author wishes to thank the members of the Plant Pathology and Entomology Laboratory, Ibaraki Agricultural Experiment Station for supplying the soils and peanuts. Special thanks are also given to Dr. Yasushi Iwasa and Mr. Morio Chiba, Department of Chemistry, National Institute of Agricultural Sciences for the advice in soil analysis.

**REFERENCES**

5) W. H. Ko & J. D. Farley: *Phytopathology* 59, 64 (1969)

要 約

Pentachloronitrobenzene に由来する Methyl Pentachlorophenyl Sulfoxide および Sulfone の土壤中および植物体中における生成

岡崎 博

ベンタクロロニトリルベンゼン (PCNB) およびその代謝物のペンタクロロフェニルアニゾール (PCTA) は土壤中において PCTA の酸化体であるメチルペンタクロロフェニルスルホキシド (PCTA-SO) およびメチルペンタクロロフェニルスルホン (PCTA-SO₂) を生成することが認められた。

殺菌塩田では PCTA-SO の生成量は非殺菌塩田の 100 分の 1 に減少し、PCTA-SO₂ の生成は認められなかったことから、その生成過程に微生物の関与していることが推察された。

PCTA-SO および PCTA-SO₂ は PCNB 処理塩田より採取した土壌からそれぞれ 0.48 ppm, 0.21 ppm 検出された。また、処理塩田に後作として栽培した落花生からはスルホキシド体、スルホン体がそれぞれ 0.006 ppm, 0.002 ppm 検出された。

PCTA-SO は PCNB 添加水耕液中で栽培したトウモロコシ植物体からも 0.007 ppm 検出されたが PCTA-SO₂ は検出されなかった。

以上の結果から、環境中において PCNB から生成した PCTA は分子内にチオエーテル結合を有する他の化合物と同様にスルホキシド体、スルホン体に酸化されることが明らかになった。