

## Mass Balance of $^{14}\text{C}$ -Pentachloronitrobenzene and Metabolites in a Closed, Aerated Soil-plant or Soil-system

M. Kamal, I. Scheunert, and F. Körte

*Gesellschaft für Strahlen- und Umweltforschung mbH, München, Institut für Ökologische Chemie, Ingolstädter Landstr. 1,  
D-8042 Neuherberg, Federal Republic of Germany*

Numerous publications are available on the fate of pentachloronitrobenzene (PCNB) in soils and plants. The mechanisms of residue losses vary considerably, depending on experimental conditions. Volatilization of unchanged PCNB was found to be the main pathway of residue loss in soil in aerated closed flasks at 25°C for 10 months (CASELEY 1968). In another study with five soils at 30°C, total residue losses were 42-60% within 18 weeks, and only <0.1% of this was carbon dioxide (IGARASHI et al. 1976a); thus, total residue losses were, also, due to volatilization. However, volatilization was unimportant in a nitrogenized closed unaerobic soil in a 40-days-experiment (MURTHY & KAUFMAN 1978). Total degradation to carbon dioxide was very small in farm and paddy soils (IGARASHI et al. 1976a) and zero in anaerobic soil (MURTHY & KAUFMAN 1978). In most of the publications available, the formation of metabolites is regarded as a major source of loss of unchanged PCNB from soils; as soil metabolites, pentachloroaniline (CHACKO et al. 1966; NAKANISHI & OKU 1969; KO & FARLEY 1969; WANG & BROADBENT 1973; BECK & HANSEN 1974; IGARASHI et al. 1976a; MURTHY & KAUFMAN 1978), pentachlorothioanisole (NAKANISHI & OKU 1969; WANG & BROADBENT 1973; BECK & HANSEN 1974; IGARASHI et al. 1976a; MURTHY & KAUFMAN 1978), pentachlorophenol (MURTHY & KAUFMAN 1978), pentachlorobenzene (BECK & HANSEN 1974), and pentachloroanisole (IGARASHI et al. 1976a) have been identified. From plants or plant enzymes, a great number of metabolites was isolated and identified (GORBACH & WAGNER 1967; KUCHAR et al. 1969; DEJONCKHEERE et al. 1975; IGARASHI et al. 1976b; BEGUM et al. 1979; LAMOUREUX & RUSNESS 1980a and b; RUSNESS & LAMOUREUX 1980; LAMOUREUX et al. 1981), including the reported soil metabolites and hydrophilic conjugates.

Mass balance studies of PCNB and conversion products using the  $^{14}\text{C}$ -labelled compound have been reported thus far only for soils (IGARASHI et al. 1976a; MURTHY & KAUFMAN 1978); balance studies in closed aerated systems are missing for soil-plant-systems. This paper reports the fate of PCNB and metabolites in a closed aerated soil-plant-system and in a soil system without plants.

## MATERIALS AND METHODS

PCNB- $^{14}\text{C}$  (specific activity: 5 mCi/mmole, radiochemical purity: > 99.5%) was synthesized in this Institute (SANDROCK et al. 1978). Inactive authentic reference samples for pentachloroaniline, pentachlorothioanisole, pentachlorobenzene, and pentachlorophenylmethylsulphoxide were commercially available or synthesized according to well-known chemical methods (BEGUM et al. 1979). Analysis of soil used: particle size distribution: sand 85.6%, silt 7%, clay 7.4%; humus 1.1%; pH 5.7.

The experiments were carried out in a glass jar, height about 20 cm, 12 cm in diameter, filled with 1 kg or 3.1 kg (dry weight), respectively, of moist soil. It was tightly closed and connected with a trap system for collecting volatile substances leaving the soil-plant-system. The trap system consisted of three tubes filled with silicon oil on glasswool to absorb lipophilic substances, a tube filled with saturated aqueous barium hydroxide solution to absorb carbon dioxide, a tube with 1-n-H<sub>2</sub>SO<sub>4</sub> to absorb volatile basic substances, and a tube with water to absorb water-soluble substances not trapped in the other tubes. Water-saturated air was drawn through the system with a pump (about 20 ml/min).

For experiment I, 2.5 mg PCNB- $^{14}\text{C}$  was dissolved in a minimum amount of acetone and the solution was mixed with 100 ml water which was thoroughly incorporated into 1 kg of air-dry soil. Eight bean seeds (Phaseolus vulgaris L.) were planted. After 16 days, when the plants were about 18 cm high, the soil was extracted in a soxhlet with hexane for 48 hours. The plants were homogenized in methanol and then extracted, also, in a soxhlet for 48 hours. The glasswool was extracted several times with cold hexane.

For experiment II, 14 mg PCNB- $^{14}\text{C}$ , dissolved in a minimum amount of acetone, was mixed with 310 ml of water which was incorporated into 3.1 kg of air-dry soil. The soil was incubated in the closed aerated system for 53 days and then extracted in a soxhlet with methanol for 48 hours. The glasswool was extracted as in experiment I.

The radioactivity in soil and plant extracts, in the absorbent liquids, and in the hexane extract of glasswool was determined by counting of aliquots in a liquid scintillation counter (Tri-Carb 3380 or 3375, Packard) using a scintillation liquid based on dioxane. Bound radioactivity left in the plant, soil, and glasswool samples after extraction was determined by combustion of aliquots in an automatic oxidizer "Oxymat" from

Intertechnique, followed by absorption of the  $^{14}\text{CO}_2$  formed in a toluene-based scintillation liquid containing phenethylamine, and counting in a liquid scintillation counter.

For quantitative determination of PCNB and conversion products in soil and plants, the extracts were evaporated to dryness with a rotary evaporator. The residues were dissolved in a few ml of methanol. Aliquots were applied on thin-layer-plates coated with silicagel G from Mackerey & Nagel (Federal Republic of Germany). After localization of radioactive zones with a scanner (Berthold/Frieseneke), the chromatograms were cut into 1-cm-fractions which were scraped off, extracted with 15-ml-portions of dioxane-based scintillation liquid, and counted in a liquid scintillation counter.

For isolation of metabolites, only the soil from experiment II was used. The total amount of concentrated extract was applied on a preparative plate coated with silicagel G. The chromatogram was developed with n-hexane. Five radioactive fractions were obtained as shown in Table I. The fractions II-V were purified by repeated thin-layer chromatography (TLC), then subjected to gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). From fraction I, four less polar components were isolated by rechromatographing in hexane and benzene, which probably had been associated with biological coextractives. They were purified by TLC with benzene and then subjected to GC and GC-MS. The remaining, very hydrophilic portion consisted of several components whose concentrations were too low for isolation and identification.

GC was carried out in a Carlo Erba gas chromatograph, detector FID, gas flow 2 ml  $\text{N}_2/\text{min.}$ ; capillary column 12.5 m long and 0.25 mm in diameter, coated with SP-2100; column temperature 60–240°C, programme 4°C/min. GC-MS was performed with a Finnigan 4023 with the same column as for GC; gas flow: 2 ml helium/min. The mass spectra were compared with those of authentic reference compounds.

#### RESULTS AND DISCUSSION

Table I presents the results of mass balance studies of PCNB- $^{14}\text{C}$  in a closed aerated soil-plant- or soil-system. The table reveals that the loss of PCNB in a soil-plant-system (Experiment I) by total mineralization is very low (0.04% under the experimental conditions used). This is in agreement with literature data from soil without plants (IGARASHI et al. 1976a; MURTHY & KAUFMAN

1978). The formation of bound residues (13.7% in soil and plants) as well as of soluble metabolites (13.5% in soil and plants) are major pathways of PCNB losses. Although only 0.06% of volatile organic substances could be trapped, the balance gap of about 15% suggests additional volatilization losses of substances which were not trapped by the equipment used. Some plant metabolites reported in a previous work (BEGUM et al. 1979), e.g. pentachlorobenzene or tetrachloronitrobenzenes, are more volatile than the parent compound.

In experiment I, the amounts of soluble metabolites were too low for GC-MS identification. Therefore, another experiment (II) was carried out only with soil, with a higher application rate and a longer exposure time, in order to obtain sufficient amounts of metabolites. The TLC-Rf-values of metabolites formed in this experiment were identical to those in soil and plants of experiment I. The TLC-fractions II-V (Table I) were found to be pentachloroaniline, unchanged PCNB, pentachlorothioanisole, and traces of pentachlorobenzene. The hydrophilic TLC-fraction I consisted of pentachlorophenylmethysulphoxide, pentachloroaniline, pentachlorothioanisole, and pentachlorobenzene which probably were associated with biological material present, and of a mixture of highly polar compounds which all were present in very low amounts and, thus, could not be identified. According to our knowledge, pentachlorophenylmethysulphoxide has not been identified as a soil metabolite of PCNB thus far.

Like in experiment I, the formation of bound residues (24.5%) and of soluble metabolites (42.5%) were major pathways of PCNB degradation in soil. Due to the longer exposure time, the conversion rates were higher than in the soil with plants. In spite of the longer exposure time, the volatilization losses in experiment II were negligible. Total degradation was < 0.01%.

In contrast to both soils where unchanged PCNB was the major TLC-fraction, in plants the very hydrophilic TLC-fraction I prevailed. According to studies with other plants (LAMOUREUX & RUSNESS 1980a), this fraction probably consists of a mixture of conjugates.

#### CONCLUSION

From these experiments, it may be concluded that conversion of PCNB to various soluble and bound metabolites is considerable but total degradation is very slow. The contribution of volatilization processes to the decrease of total PCNB-derived residues varies with environmental conditions.

TABLE I. Mass Balance of PCNB- $^{14}\text{C}$  in a Closed Aerated  
Soil-Plant- or Soil-System  
(in % of initially applied  $^{14}\text{C}$ )

Fraction	Experiment I (soil with beans) 2.5 $\mu\text{g/g}$ dry soil, 16 days	Experiment II (soil) 4.5 $\mu\text{g/g}$ dry soil, 53 days
$^{14}\text{CO}_2$ trapped	0.04	0.01
Volatile organic substances, trapped	0.06	0.01
<b>Soil:</b>		
unextractable $^{14}\text{C}$	12.63 (hexane)	24.53 (methanol)
extractable $^{14}\text{C}$ , sum	67.37	79.02
TLC*-fraction I	2.76	16.12 PCPMS 2.60 PCA 4.80 PCTA 4.88 QCB 2.56 unidenti- fied 1.28
TLC*-fraction II (Rf 0.12) = PCA	3.10	13.91
TLC*-fraction III (Rf 0.42) = PCNB	57.20	36.51
TLC*-fraction IV (Rf 0.57) = PCTA	4.31	12.40
TLC*-fraction V (Rf 0.70) = QCB	< 0.01	0.08
total $^{14}\text{C}$ in soil	80.00	103.55
<b>Beans:</b>		
unextractable $^{14}\text{C}$	1.05	
extractable $^{14}\text{C}$ , sum	4.21	
TLC*-fraction I (Rf 0)	2.90	
TLC*-fraction II (Rf 0.21)	0.33	
TLC*-fraction III (Rf 0.42)	0.86	
TLC*-fraction IV (Rf 0.57)	0.12	

TABLE I continued

TLC*-fraction V (Rf 0.70)	< 0.01
total $^{14}\text{C}$ in beans	5.26
Total $^{14}\text{C}$ recovered	85.36
	103.56

\* solvent: hexane

Abbreviations used: PCPMS = pentachlorophenylmethylsulphoxide; PCA = pentachloroaniline; PCTA = pentachlorothioanisole; QCB = pentachlorobenzene

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