

AEROBIC BIODEGRADATION OF [<sup>14</sup>C]3-CHLORO-*P*-TOLUIDINE  
HYDROCHLORIDE IN A LOAM SOILRONALD J. SPANGGORD,† G. ROSS GORDON,† ROBERT I. STARR\*‡ and DONALD J. ELIAS‡  
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**Abstract**—Degradation of the pesticide 3-chloro-*p*-toluidine hydrochloride (CPTH) occurred in a loam soil when applied at concentrations of 3.5 and 35  $\mu\text{g/g}$ . The compound degraded according to pseudo-first-order kinetics, with a calculated rate constant of  $2.74 \times 10^{-2} \text{ h}^{-1}$ , at a soil temperature of 22°C; this rate constant yielded a half-life of 25 h. The loss of radiolabeled CPTH from soil was suggested to be controlled by both irreversible binding to the soil colloids and microbial transformation. Mineralization of the radiolabeled CPTH was interpreted as involving two zero-order kinetic rates; an initial rate of carbon dioxide release was estimated to be  $0.33\% \text{ d}^{-1}$  (half-life of 152 d), followed by a slower rate of  $0.07\% \text{ d}^{-1}$ , which resulted in a half-life of 718 d. Approximately 13% of the radiolabeled CPTH that was applied to soil at 3.5  $\mu\text{g/g}$  was mineralized to [<sup>14</sup>C] carbon dioxide during the 99-d incubation period. A primary metabolite was identified as *N*-acetyl-3-chloro-*p*-toluidine (ACPTH); this metabolite reached a maximum concentration at the 1-d sampling period, and degraded with a pseudo-first-order rate constant of  $2.67 \times 10^{-2} \text{ h}^{-1}$ ; the half-life for ACPTH was calculated to be 26 h. When CPTH was applied to soil at 35  $\mu\text{g/g}$ , the compound was also mineralized in soil by a similar metabolic pathway to that observed at the lower concentration. However, the rate of mineralization was slower, which suggests that elevated soil concentrations of CPTH may affect the viability of certain microorganisms.

**Keywords**—Biodegradation Metabolism Pesticide CPTH Soil

## INTRODUCTION

The compound 3-chloro-*p*-toluidine hydrochloride (CPTH) has been demonstrated to be an effective and unique pesticide because of its selective high toxicity to most pest bird species and low-to-moderate toxicity to the majority of predatory birds and mammals, and a lack of known secondary hazards when used in baits. The mode of action of CPTH in starlings has been suggested to be that of a nephrotoxin [1]. The acute toxicity (oral LD<sub>50</sub>) of the avicide to starlings is reported to be between 4 and 5 mg/kg of body weight [2]. The primary metabolites of CPTH are indicated to be of low toxicity to birds and mammals [3]. Under field conditions where the avicide is applied to soils as a seed or pellet bait, some of the compound is undoubtedly solubilized and leached into the soil; however, limited data are currently available regarding the fate of this minor-use pesticide in soils. To provide additional environmental data and to comply with U.S. Environmental Protection Agency (EPA) reregistration information requirements, an aerobic soil biodegradation study was conducted at our laboratory.

As reported by Laut (S. Laut, unpublished report), CPTH has an estimated pK<sub>a</sub> of 3.9 for protonation of the amine group. Thus, in moist soils at pH values greater than 4.8, more than 90% of the chemical will be in the free amine (chloroaniline) form as 3-chloro-*p*-toluidine (CPT); conversely, the ammonium ion form (CPTH) will be the major compound present only in acidic soils below pH 3.

Bollag et al. [4] reported that the behavior of chloroanilines in soil was complex, due to competitive reactions involving both sorption and microbial degradation. Smith and Briggs [5] evaluated the persistence of the free aniline compound CPT in

soil at concentrations that ranged from 2.5 to 50  $\mu\text{g/g}$ ; at the 72-h sampling point, the compound could not be detected. At high concentrations, coupling of the aniline compound was observed, which resulted in the formation of azo (I) and quinone (II) products, as illustrated in Figure 1. These products were not detected when the chloroaniline compound was applied to soil at concentrations below 5  $\mu\text{g/g}$ . Using the pesticide propanil (3',4'-dichloropropionanilide), Bartha [6] determined that about 73% of a 5- $\mu\text{g/g}$  application was sorbed to soil, whereas 54% became bound when 500  $\mu\text{g/g}$  of the chemical were applied; thus, concentration may affect the degree of soil sorption.

Preliminary biodegradation research conducted at our laboratory using both sterile and nonsterile loam soil demonstrated that the pesticide disappeared rapidly from soil treated with 3  $\mu\text{g/g}$  of CPTH (data and HPLC profiles not shown). The amount of compound that was extractable in nonsterilized soil was degraded to low detectable levels within 3 d. A metabolite was formed that also transformed with time. In soil that was sterilized by autoclaving for 1 h on three successive days, a similar loss of CPTH was observed as in the nonsterilized soil. As observed by Bollag et al. [4], Smith and Briggs [5], and Kearney and Plimmer [7], these data suggested that the initial loss of CPTH was related to soil sorption of the free amine form of the pesticide (CPT).

The study presented in this paper describes the aerobic biodegradation of [<sup>14</sup>C]CPTH in a loam soil treated with 3.5 and 35  $\mu\text{g/g}$  of the radiolabeled pesticide.

## MATERIALS AND METHODS

*Test chemicals*

Nonradiolabeled CPTH (Purina Mills, Bridgeton, MO, USA), purity of 99%, and uniformly ring-labeled [<sup>14</sup>C]CPTH (Sigma

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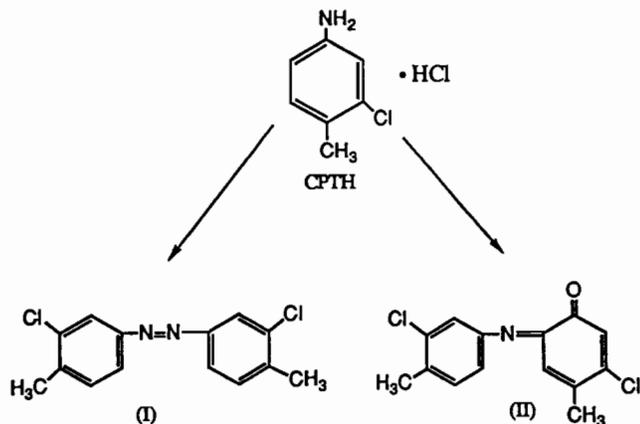


Fig. 1. Coupling reactions observed when the free aniline compound (CPT) was added to soil at various concentrations [5].

Chemical Company, St. Louis, MO, USA), specific activity of 2.25 mCi/mmol ( $8.3 \times 10^7$  Bq/mmol), were used; purity of the radiolabeled compound was >98% as determined by HPLC. The test chemicals were stored at  $-20^\circ\text{C}$  until used.

#### Soil selection and preparation

The loam soil selected for the biodegradation research was collected in Menlo Park, California, within the grounds of SRI International, because soil from application sites was unavailable. Following removal of approximately the upper 10 cm of surface soil, the material was passed through a 2-mm sieve to remove large soil particles and extraneous debris. The soil was used within 10 d after collection. Characterization data for the test soil were as follows: texture, loam; sand, silt, and clay, 48, 42, and 10%, respectively; organic matter, 8.2%; pH, 6.5; and cation exchange capacity, 25.3 meq/100 g. These analyses were performed under GLP by Agvise Laboratories, Northwood, North Dakota.

#### Incubation apparatus

The soil incubation apparatus involved the use of 250-ml glass Bartha biometer flasks [8]. A 1 N KOH solution (10 ml) was placed in the side arm of the flasks and used as the trapping solution for [<sup>14</sup>C]CO<sub>2</sub>. The neck of each flask contained a rubber stopper that held a small chromatographic column (10 mm i.d.) that was equipped with a stopcock that remained closed except during the additions of fresh KOH to the side arm or of air to the system. A glass wool plug was used in the column, together with sufficient Ascarite® (CO<sub>2</sub> absorbent), which was added to form a column about 5 cm in height. At appropriate sampling periods, the KOH solution was removed for radiochemical analysis using an attached syringe; fresh KOH (10 ml) was then added to the flask side arm. Using the syringe, the soil flasks were aerated by pushing 10 ml of air into the side arm after adding the KOH solution. The biodegradation study was conducted in accordance with methodology published by the EPA [9].

#### Soil treatment

Fifty-gram quantities of the air-dried loam soil were weighed into the 250-ml flasks; deionized water was added in 2- to 3-ml increments (swirling the flasks between additions), until a final moisture content approximating 68% of field capacity was obtained. The soil flasks were incubated at room temperature for 5 d with the tops open to the atmosphere to assure reactivation

of the microbial populations; moisture evaporation was monitored daily (weight loss) during this period. Radiolabeled CPTH (2.25 mCi/mmol;  $8.3 \times 10^7$  Bq/mmol) was dissolved in 70% acetonitrile in deionized water at a concentration of 878 µg/ml. For the 3.5 µg/g soil treatment, the samples were uniformly surface-treated with 200 µl (about 176 µg) of the [<sup>14</sup>C]CPTH solution, while mixing the soil with a glass stirring rod; the stirring rod was rinsed with about 2.8 ml of deionized water, which was equal to the mean soil moisture evaporation that was recorded during the pretreatment 5-d incubation period. The 35-µg/g treatment level was achieved by adding 200 µl of an acetonitrile-water solution of unlabeled CPTH containing 7873 µg/ml, in addition to the same volume (200 µl) of [<sup>14</sup>C]CPTH used for the 3.5-µg/g concentration; this procedure resulted in about 1,751 µg of combined unlabeled and radiolabeled CPTH (2.23 µCi;  $8.25 \times 10^4$  Bq) per 50 g of soil, for an overall soil concentration of 35 µg/g.

Sterilized soil used in a preliminary study was prepared by autoclaving for 1 h on three successive days, using methodology reported by Oepen et al. [10].

#### Sampling intervals

After treatment and thorough mixing of the soil by stirring, the biometer sample flasks were maintained in a laboratory cabinet under dark conditions at a temperature of  $21.7 \pm 1.4^\circ\text{C}$  during the 99-d incubation period.

In anticipation of a rapid initial loss of the radiolabeled chemical from the soil, four sampling periods were chosen (3.5-µg/g treatment level) to evaluate residual soil [<sup>14</sup>C]CPTH, radiolabeled metabolites, and mineralized [<sup>14</sup>C]CPTH as CO<sub>2</sub>; time of treatment ( $t = 0$ ) and 1, 2, 3, and 4 d following treatment; thus, the study involved 10 sample flasks that were analyzed in duplicate on days 0, 1, 2, 3, and 4, with single sample recoveries performed on days 38 and 99 (Table 1). The continued mineralization of the radiolabeled pesticide to [<sup>14</sup>C]CO<sub>2</sub> was then followed by sampling the soil flasks 8, 15, 22, 29, 36, 43, 50, 57, 64, 81, and 99 d after treatment.

At the elevated 35-µg/g treatment level, three sampling periods were chosen to evaluate residual soil [<sup>14</sup>C]CPTH and radiolabeled metabolites: 1, 3, and 85 d following treatment. A total of 12 sampling periods were selected to determine the degree of mineralization of the radiolabeled compound: 1, 3, 4, 8, 15, 22, 29, 36, 43, 50, 67, and 85 d after treatment. At each sampling interval, one treated sample and a control were analyzed for residual CPTH, metabolites, and/or [<sup>14</sup>C]CO<sub>2</sub>. The flasks were weighed at each sampling point to monitor soil moisture loss.

#### Analytical methods

The KOH trapping solutions were analyzed for [<sup>14</sup>C]CO<sub>2</sub> and radiolabeled volatile metabolites; each 50-g soil sample was extracted for total residual radioactivity as described below.

**Soil extraction procedure.** After transferring the soil to a 250-ml polypropylene screw-cap bottle, residual soil was removed from the sample flask with several 10-ml aliquots of 80% acetonitrile-water solution. The soil was then extracted for 10 min using a mechanical shaker maintained in a cold room at about  $4^\circ\text{C}$ , followed by centrifuging the bottle under reduced temperature for 10 min at 3,000 g. The extract was decanted into a graduated cylinder, the volume recorded, and the extraction was then repeated in a sequential manner using 50 ml each of 80% acetonitrile-water, then methanol, followed by 1 N NaOH. After the last extraction, the residual soil was removed

Table 1. Recovery of [<sup>14</sup>C]CPTH from a loam soil treated with 3.5 μg/g of the parent compound

Incubation period (d)	Percent recovery of radioactivity (mean values) <sup>a</sup>							
	Extractable				Total	Volatile <sup>b</sup>	Bound to soil	Total recovery
Acetonitrile (1st)	Acetonitrile (2nd)	MeOH	NaOH					
0	52.64	18.14	5.50	4.28	80.56	NA <sup>c</sup>	18.37	98.93 ± 0.64
1	13.02	6.12	2.10	7.84	29.08	1.38	57.39	87.85 ± 1.90
2	8.76	3.94	1.31	6.70	20.72	2.02	67.44	90.18 ± 3.10
3	6.84	3.22	1.08	6.46	17.60	2.65	72.39	92.64 ± 0.01
4	6.17	2.96	1.10	7.36	17.59	3.20	70.33	91.12 ± 0.50
38	2.77	1.30	0.77	5.28	10.12	8.83	82.72	101.70
99	2.63	1.18	0.51	6.55	10.87	12.37	70.79	94.03

<sup>a</sup> Duplicate values except for days 38 and 99, which were single samples.

<sup>b</sup> Volatile fraction contained only [<sup>14</sup>C]CO<sub>2</sub>.

<sup>c</sup> NA = not applicable.

from the bottle by adding 10 ml of methanol, suspending the soil particles with vigorous shaking of the bottle by hand, followed by decanting the soil suspension into a large tared polypropylene weighing boat; this methanol treatment was repeated four additional times, after which the soil was allowed to dry under a hood for 48 to 72 h. After weighing the dry soil sample, it was transferred to a glass mortar, ground to a fine powder, and then stored at -16°C until it was combusted for residual [<sup>14</sup>C] activity.

**Extractable radioactivity.** Radioactivity present in the soil extracts was determined using liquid scintillation counting (LSC) techniques. A quench curve was prepared by adding 50 μl of a radiochemical standard ([<sup>14</sup>C] Spec-heck<sup>®</sup>, 9.17 × 10<sup>5</sup> dpm/ml, Packard Instrument Co., Downers Grove, IL, USA) to 5 ml of Ecolite<sup>®</sup> scintillation fluid (ICN, Chicago, IL, USA). Between 10 and 80 μl of a dilute methyl orange quench agent were then added, to yield counting efficiencies that ranged from 94% (no quench agent) to 65% (80 μl of methyl orange), using a Beckman Model LSC-7000 (Fullerton, CA, USA). Regression analysis of the counting efficiency versus the instrument quench factor for each standard sample provided the regression equation for calculating the counting efficiency of unknown samples.

After adding 0.5 or 0.2 ml of the organic and NaOH extracts, respectively, to 5 ml of the scintillation cocktail, duplicate samples were counted using LSC. All instrument counts (cpm) were corrected to dpm using the regression equation.

**Residual soil radioactivity.** Bound [<sup>14</sup>C] residues were combusted using a Model B306 Packard Tricarb oxidizer (Packard Instrument Co., Downers Grove, IL, USA). Soil samples were weighed in duplicate (500 mg) in paper combustion cones containing 250 mg of powdered cellulose. Upon combustion, the resulting [<sup>14</sup>C]CO<sub>2</sub> was flushed into glass scintillation vials containing 10 ml of the Carbo-Sorb-E<sup>®</sup> trapping compound and 14 ml of Permafluor E+<sup>®</sup> scintillation fluid. The capped vials were then counted for 2 min using LSC.

A quench curve was prepared by combusting several 500-mg samples of control loam soil in a similar manner to that described above, followed by the addition of a quench agent and a [<sup>14</sup>C] standard. The typical soil combustion efficiency was 93.3% (triplicate analyses) for standards added to soil at the 20-nCi level (7.4 × 10<sup>2</sup> Bq) and 101% at the 2-nCi level (7.4 × 10<sup>1</sup> Bq). Standards were analyzed each day that a soil combustion was performed.

**Mineralized [<sup>14</sup>C]CPTH and other volatile products collected in KOH trap.** Volatile [<sup>14</sup>C]-labeled compounds, that were cap-

tured in the KOH trapping solution during the soil incubation period, were determined in duplicate by adding 0.25 ml of the KOH solution, plus 50 μl of 50% acetic acid, to 5 ml of Ecolite +<sup>®</sup> scintillation fluid. Formation of [<sup>14</sup>C]CO<sub>2</sub> was evaluated by adding 0.7 ml of 0.2 N BaCl<sub>2</sub> to 0.7 ml of the trapping solution in a 1.5-ml polypropylene microfuge tube; after centrifuging the tube 4 min at 16,000 g, 0.5 ml of the supernatant liquid was added to 5 ml of scintillation fluid, together with 50 μl of 50% acetic acid. Sample counting efficiency was determined using a quench curve as described previously. Total radioactivity in terms of [<sup>14</sup>C]CPTH equivalents was calculated for the samples by counting the solutions before and after the radiolabeled carbonate was precipitated; thus, the difference between the before and after counts represented the fraction of the [<sup>14</sup>C]CPTH soil application that was mineralized.

#### Metabolite identification

**Synthesis of *N*-acetyl-CPTH.** An authentic sample of *N*-acetyl-3-chloro-*p*-toluidine (ACPTH) was synthesized using the standard procedures reported by Vogel [11]. The melting point of the resulting product (84–85°C) was consistent with that reported by Schofield (83°C) [12].

**Thin-layer chromatography of soil extracts.** Aliquots (1.0–6.5 ml) of the first acetonitrile extract of loam soil samples that had been treated with 35 μg/g of [<sup>14</sup>C]CPTH and incubated for various periods were acidified with 10 μl of concentrated HCl and evaporated to dryness under nitrogen; the resulting residue was dissolved in 500 μl of methanol. Total radioactivity was determined by LSC (25 μl), with the remaining sample applied as an approximate 2.5-cm streak to a silica gel 60 plate (20 × 20 cm) containing a fluorescent indicator (E. Merck, Darmstadt, Germany). Reference spots of [<sup>14</sup>C]CPTH and unlabeled ACPTH were coapplied. The compound ACPTH was identified as a metabolite in a previous fish bioaccumulation–metabolism study [13]. After development of the plate with chloroform: ethyl acetate: glacial acetic acid (75:20:5 v/v), the air-dried plate was viewed under UV light (254 nm). An autoradiogram was then prepared by exposing the plate for 4 d to X-OMAT AR<sup>®</sup> film (Eastman Kodak, Rochester, NY, USA).

**HPLC separation of soil extracts.** Chromatographic separations were achieved using a Hewlett-Packard Model 1084 B HPLC equipped with a gradient controller and a variable UV detector set at 241 nm and an automatic sample injector. Separations were performed using a reverse-phase C-18 column (4.6 × 250 mm, Beckman Instruments, Riverside, CA, USA),

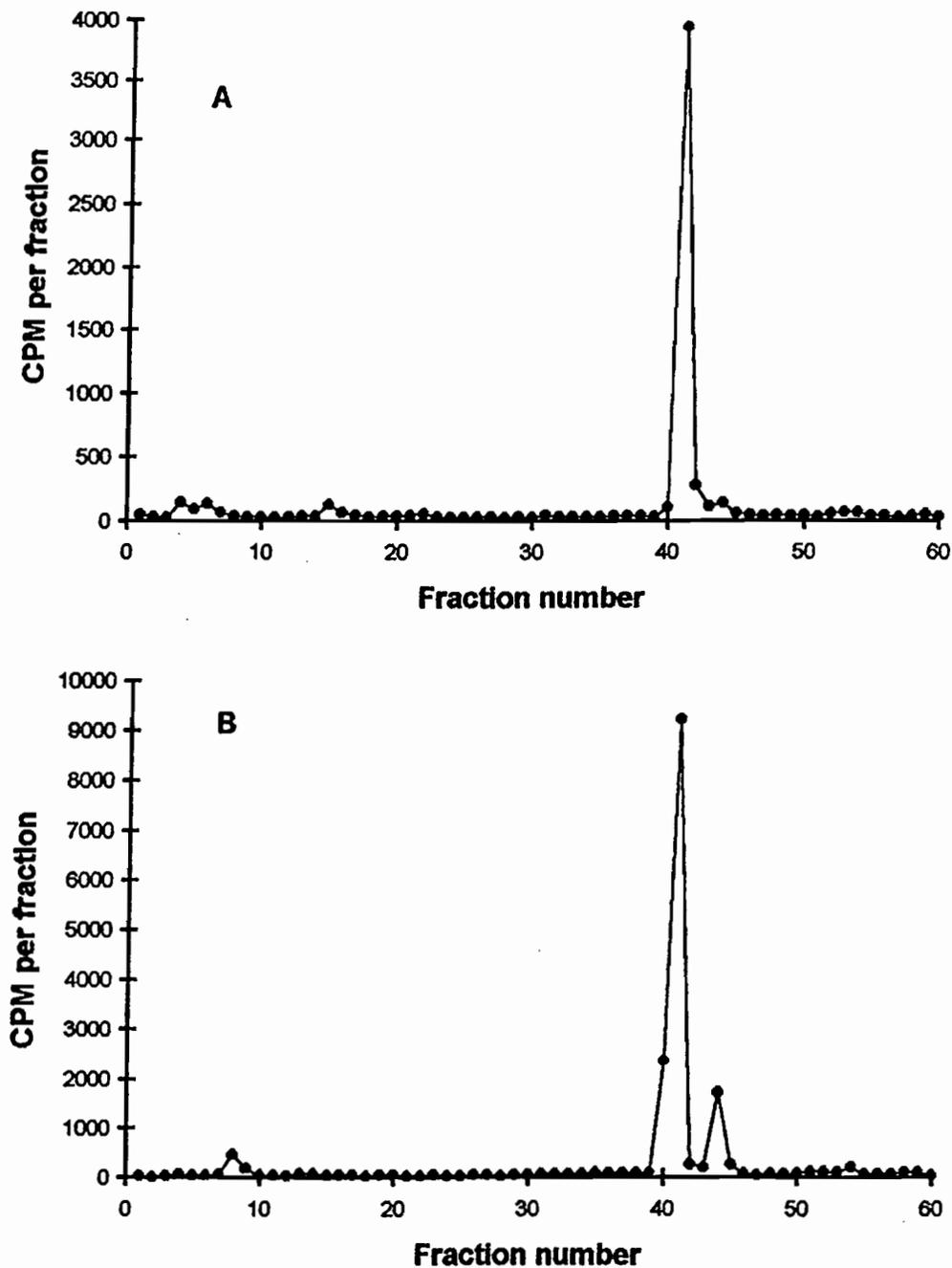


Fig. 2. Qualitative HPLC profiles of radioactivity in acetonitrile extracts of soil treated with 3.5  $\mu\text{g/g}$  [ $^{14}\text{C}$ ]CPTH at  $t = 0$  (A, 0.5 ml of sample extract) and  $t = 24$  h (B, 1.0 ml of sample extract). Fractions 41 and 43 are CPTH and ACPH, respectively.

with the column maintained at 35°C. The mobile phase was either 50 or 60% methanol at 1.0 ml/min when isocratic runs were made; a gradient that started at 10% methanol and increased to 80% methanol over 60 min was used for the more complex separations.

Because the initial 80% acetonitrile extracts contained the most radioactivity, these extracts were subjected to HPLC. The general preparation procedure that was used prior to separation by HPLC involved acidification of the extracts using concentrated HCl; evaporation of the extracts to dryness under nitrogen; dissolving the residues in 50% methanol; and centrifuging the microfuge tubes containing the dissolved residues at 16,000 g. The supernatant solutions were then transferred to microinjection vials, and aliquots of these solutions were chromatog-

raphed, collecting fractions at 1-min intervals directly into scintillation vials; radioactivity of the samples was then determined using LSC.

#### Data analysis

*Half-life estimation for loss of the parent chemical.* The loss rate of CPTH due to microbial degradation is dependent upon the chemical concentration ( $C$ ) and the microbial population ( $X$ ). If the microbial population is assumed to remain constant over the duration of the study, a pseudo-first-order rate expression can be developed as in Equation 1, where  $k_b$  is the pseudo-first-order rate constant.

$$-dC/dt = k_b C \quad (1)$$

Integration of Equation 1 yields Equation 2, where  $C_0$  and  $C_t$  are the concentration of the test chemical at time 0 and time  $t$ .

$$\ln C_0/C_t = -k_b t \quad (2)$$

A plot of  $\ln C_0/C_t$  versus  $t$  will yield a straight line with a slope of  $k_b$ . Once the slope is determined, the half-life ( $t_{1/2}$ ) of CPTH can be calculated from Equation 3.

$$t_{1/2} = \ln 2/k_b \quad (3)$$

Straight lines were obtained using linear regression (Cricket Graph®, Cricket Software, Melvern, PA, USA).

**Mineralization rate of CPTH.** The rate at which the parent compound was mineralized to  $\text{CO}_2$  was considered to be a zero-order rate process, independent of the CPTH initial application. This was based upon the complexity of the initial irreversible sorption process and the release of  $\text{CO}_2$  from humic-bound material as described by Brunner and Focht [14]. Thus, the rate of formation of  $\text{CO}_2$  with respect to time was considered constant, as in Equation 4, where  $k_c$  is the zero-order rate constant for the formation of  $\text{CO}_2$ .

$$\begin{aligned} d\text{CO}_2/dt &= k_c \\ \% \text{CO}_2/t &= k_c \end{aligned} \quad (4)$$

If the rate constant is calculated in units of  $\% \text{ d}^{-1}$ , the half-life for mineralization of CPTH can be determined by Equation 5.

$$t_{1/2} = 50/k_c \quad (5)$$

## RESULTS

### Loss of the parent compound from soil treated with $3.5 \mu\text{g/g}$ of [ $^{14}\text{C}$ ]CPTH

The radiolabeled CPTH readily degraded in the treated loam soil, with a primary metabolite identified as ACPH, which was observed to occur at the 1-d sampling period (Fig. 2). Loss of [ $^{14}\text{C}$ ]CPTH from the loam soil occurred according to pseudo-first-order kinetics (Table 1 and Fig. 3); analysis of the data yielded a rate constant of  $2.74 \times 10^{-2} \text{ h}^{-1}$ , from which a half-life of 25.3 h was calculated. One day following treatment, the radiolabeled CPTH (i.e., CPT) was observed to sorb rapidly and strongly to the loam soil, with about 57% bound and not removed by successive extractions of acetonitrile (two) followed by methanol and a final sodium hydroxide extraction; this loam soil had a high organic matter content (8%) and cation exchange capacity. As illustrated in Table 1, the quantity of [ $^{14}\text{C}$ ]CPTH extracted from the soil using acetonitrile decreased over time, with about 55  $\mu\text{g}$  (0.3%) of the original application extracted at 0 d, which then decreased to about 5  $\mu\text{g}$  at 4 d (Table 2). During this period the quantity of radiolabeled metabolite (ACPTH) detected was 0.8  $\mu\text{g}$  at day 0, increasing to 5.6  $\mu\text{g}$  at day 1, and then decreasing to 0.9  $\mu\text{g}$  at day 4 (Table 2). Total recovery of [ $^{14}\text{C}$ ]CPTH and radiolabeled metabolites during the entire 99-d incubation period ranged from about 88 to 102% (Table 1).

Moisture loss from the soil flasks, monitored by weighing the flasks at each sampling period, was found to be negligible during the 99-d study (data not shown); moreover, some weight gain was observed due to moisture condensing in the Ascarite tubes.

**Metabolite formation and loss.** The radiolabeled components collected using HPLC were concentrated in fractions 41 and 43, as illustrated in Figure 2. Fraction 41 represents the parent CPTH and fraction 43 contains the primary radiolabeled metabolite, which was identified as ACPH by cochromatography

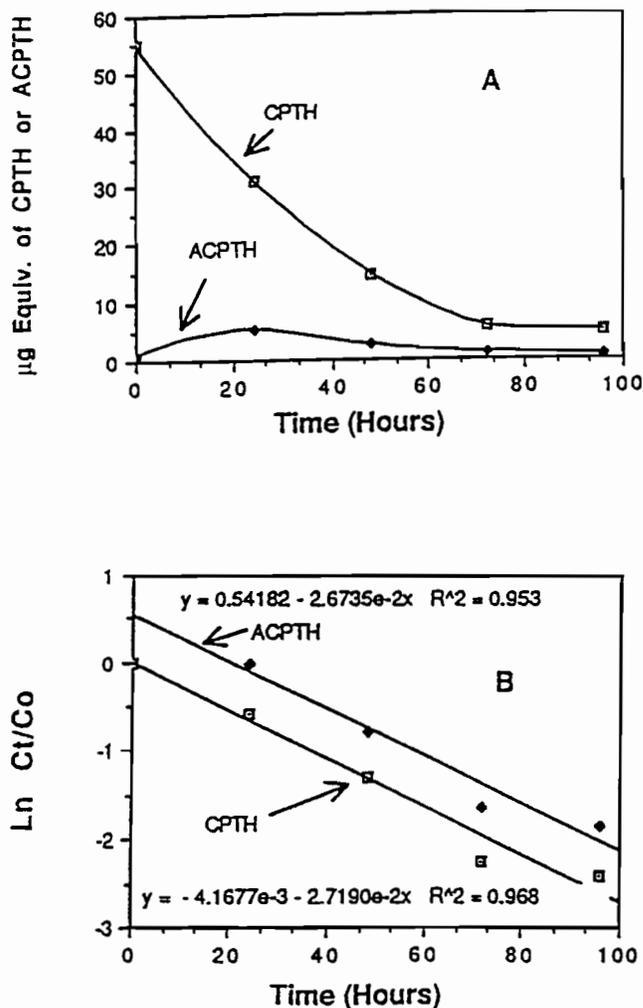


Fig. 3. Loss of [ $^{14}\text{C}$ ]CPTH and formation and loss of [ $^{14}\text{C}$ ]ACPTH in a loam soil treated with  $3.5 \mu\text{g/g}$  CPTH (A) and pseudo-first-order rate plots for CPTH and ACPH (B).

using HPLC. This product reached a maximum concentration at 1 d and then degraded at a rate similar to that determined for [ $^{14}\text{C}$ ]CPTH (Table 2 and Fig. 3). The rate of formation of ACPH was estimated to be  $0.2 \mu\text{g h}^{-1}$  (two data points), with the pseudo-first-order rate constant for decline calculated to be  $2.67 \times 10^{-2} \text{ h}^{-1}$ , which was essentially the same as the loss rate of CPTH in the loam soil ( $2.74 \times 10^{-2} \text{ h}^{-1}$ ). The small amount of radioactivity detected in fraction 8 (Fig. 2) represents an unidentified polar metabolite.

Table 2. Quantity of [ $^{14}\text{C}$ ]CPTH and [ $^{14}\text{C}$ ]ACPTH in acetonitrile extracts from a loam soil treated with  $3.5 \mu\text{g/g}$  of the parent compound

Incubation period (d)	CPTH (mean $\mu\text{g}$ ) <sup>a</sup>	ACPTH (mean $\mu\text{g}$ ) <sup>a</sup>
0	54.8	0.8
1	31.1	5.6
2	14.9	2.6
3	5.7	1.1
4	4.9	0.9

<sup>a</sup> Values represent total quantity of these radiolabeled compounds (in micrograms) found in the first acetonitrile extract of each soil sample; data were quantified by collecting each fraction from the HPLC and then performing liquid scintillation counting of the entire fraction.

Table 3. Recovery of [<sup>14</sup>C]CPTH from a loam soil treated with 35 μg/g of the parent compound

Incubation period (d)	Percent recovery of radioactivity*			
	Extractable	Volatile	Bound to soil	Total recovery
1	40.00	0.61	53.73	94.34
3	27.45	1.42	56.34	85.21
85	11.84	6.64	72.40	90.88

\* Single samples.

**Mineralization rate of [<sup>14</sup>C]CPTH.** Approximately 13% of [<sup>14</sup>C]CPTH that was applied to soil at 3.5 μg/g was mineralized to [<sup>14</sup>C]CO<sub>2</sub> during the 99-d incubation period. The mineralized product was identified as radiolabeled CO<sub>2</sub> by precipitating the radiolabeled carbonate collected in the KOH trap using BaCl<sub>2</sub>. All of the trapped radioactivity was attributed to [<sup>14</sup>C]CO<sub>2</sub>. The cumulative release of [<sup>14</sup>C]CO<sub>2</sub> from soil treated with 3.5 μg/g of radiolabeled CPTH over time is illustrated in Figure 4. Moreover, these data can be interpreted as two zero-order kinetic formation processes (Fig. 4). Mineralization of the radiolabeled CPTH occurred at a rate of 0.3% d<sup>-1</sup> up to d 15; a half-life of 152 d was then calculated for the mineralization of CPTH.

#### Loss of the parent compound from soil treated with 35 μg/g of [<sup>14</sup>C]CPTH

After treating several soil flasks with 35 μg/g of the radiolabeled compound, the soil flasks were incubated under the same conditions as those used for the 3.5 μg/g concentration. Using HPLC with UV detection, the breakdown of CPTH was followed; moreover, as observed at 3.5 μg/g, rapid formation of the metabolite occurred at 1 d. Also, the metabolite degraded rapidly (data and HPLC profiles not shown).

Identification of the metabolite was further confirmed by TLC analysis of extracts from loam soil samples that had been treated with 35 μg/g [<sup>14</sup>C]CPTH and incubated. An autoradiogram prepared from a developed TLC plate demonstrated the presence of ACPTH at days 1 and 3 of the incubation period; this radiolabeled metabolite was absent at the 85-d sampling period using the R<sub>f</sub> of the authentic compound for comparison. The total recovery of [<sup>14</sup>C]CPTH for single soil samples monitored at days 1, 3, and 85 is summarized in Table 3. The radiolabeled CPTH mineralized over time, with the cumulative production of [<sup>14</sup>C]CO<sub>2</sub> illustrated in Figure 5. However, the cumulative production of radiolabeled CO<sub>2</sub> over time (85 d) as a percentage of the applied [<sup>14</sup>C]CPTH was lower (7%) than the value obtained (13%) at the 3.5-μg/g treatment level over a slightly longer 99-d incubation period.

#### DISCUSSION

This study demonstrated that CPTH was readily metabolized by soil microorganisms, with loss of the parent compound from a loam soil occurring according to pseudo-first-order kinetics; a half-life of 25 h was calculated for the compound. Moreover, the pesticide was found to sorb rapidly and strongly to soil colloids and could not be extracted effectively using acetonitrile and methanol or 1 N sodium hydroxide; after a 1-d incubation period, about 57% of the radiolabeled CPTH was determined to be bound to the soil, which contained 8% organic matter and had a clay content and CEC of 10% and 25.3 meq/100 g, respectively. This finding was confirmed in preliminary studies with sterilized soil, confirming the competitive abiotic process

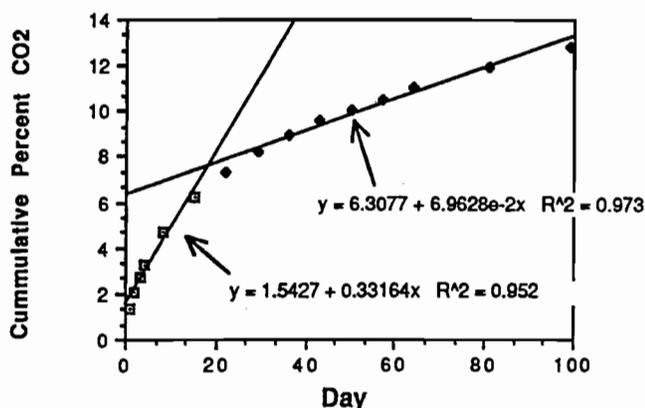
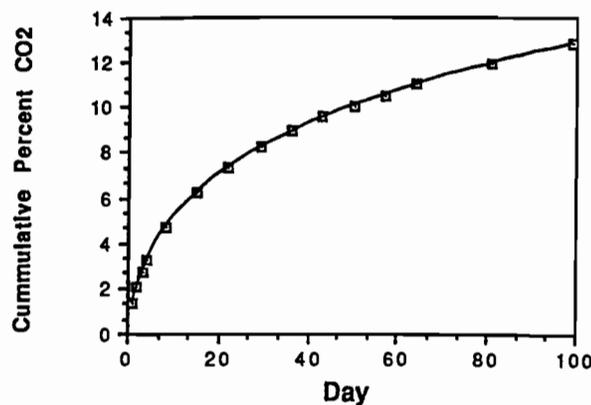


Fig. 4. Formation of [<sup>14</sup>C]CO<sub>2</sub> as a function of time in a loam soil treated with 3.5 μg/g [<sup>14</sup>C]CPTH (A); zero-order rate approximations for mineralization of CPTH (B).

leading to the loss of CPTH. The strong sorption of various aromatic amines, similar in structure to CPTH, has been previously reported [4,5,7]. Thus, dissipation of CPTH from soil appears to be the result of both soil sorption and microbial processes.

The initial and dominant biodegradation product was determined to be ACPTH, which was evident within 1-d of incubation. The metabolic transformation step is illustrated in Figure 6. Inasmuch as this reaction occurred rapidly upon addition of CPTH to the soil, a lag phase was not observed. Moreover, the

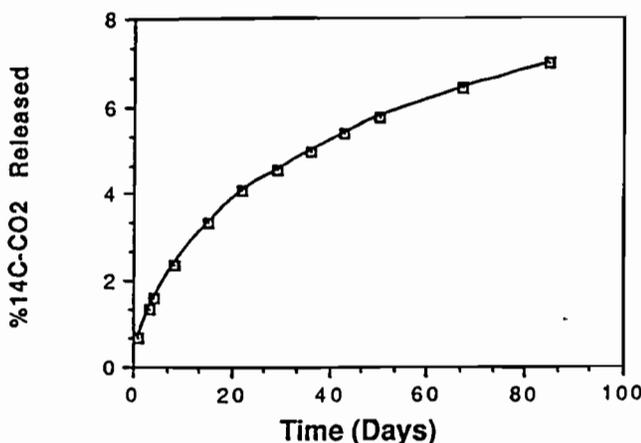


Fig. 5. Formation of [<sup>14</sup>C]CO<sub>2</sub> as a function of time in a loam soil treated with 35 μg/g [<sup>14</sup>C]CPTH.

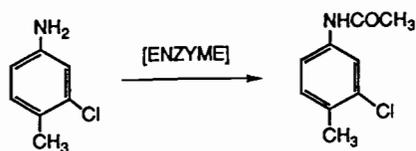


Fig. 6. Primary metabolic transformation step for CPTH in soil.

primary metabolite (ACPTH) was rapidly degraded and/or sorbed to the soil colloids, and disappeared at a rate similar to that observed for CPTH.

Mineralization of the radiolabeled CPTH (release of [ $^{14}\text{C}$ ]CO $_2$ ) was observed to occur after 1 d of incubation, which suggests that soil microbial populations can utilize CPTH as an energy source. Mineralization of CPTH appeared to occur at two different rates: an initial rate of CO $_2$  release of 0.33% d $^{-1}$ , followed by a slower rate of 0.07% d $^{-1}$  (Fig. 4). When the soil concentration of CPTH was increased from 3.5 to 35  $\mu\text{g/g}$ , the release of radiolabeled CO $_2$  decreased, which suggests that the higher pesticide concentration may have had a toxic effect upon selected microbial populations.

Smith and Briggs [5] reported that condensation products occurred when CPT was applied to soil at high concentrations (50  $\mu\text{g/g}$ ), but were not detected at concentrations below 5  $\mu\text{g/g}$ . The primary transformation step was reported to involve hydroxylation of the amine group; the reactive intermediate product then reacted with itself to form azoxy and azo derivatives. In our research, certain unidentified products were detected using TLC; however, these products were minor compared to the quantity of ACPTH that formed and did not warrant further investigation. It is possible that *N*-hydroxylation of CPTH occurred at the elevated concentration, and this was then rapidly sorbed to the humic soil components as the principal bound product; however, regardless of the CPTH soil binding mechanism, mineralization of the compound occurred.

Stockinger et al. [15] described the mineralization of CPT in terms of chloride and ammonium ion release using a pure strain of *Pseudomonas cepacia* that was isolated from soil. These authors proposed an ortho-cleavage mechanism for degradation of the parent compound, after they isolated two 1,2-dioxygenase enzymes and performed certain oxygen-uptake experiments. This type of enzymatic cleavage would be anticipated to occur in order to produce short-chained carboxylic acids that will ultimately form carbon dioxide. These results provide further

evidence for the utilization of CPTH by soil bacteria under aerobic conditions.

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