Identification of Degradation Products of the Avicide 3-Chloro-p-toluidine Hydrochloride in Louisiana Rice Fields

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An investigation of the migration of 3-chloro-p-toluidine hydrochloride (CPTH) on treated rice baits to soils in Louisiana rice fields was completed. The persistence of free CPTH in these soils was evaluated with field and laboratory experiments. These soils were also screened for the presence of CPTH degradation products. Soils treated with CPTH-fortified rice bait and aqueous solutions of CPTH were exposed under actual field conditions for a 13-day period. Control soil samples were also treated with CPTH and incubated under simulated field conditions in an environmental chamber. Soil samples were then screened for CPTH residues and degradation products by HPLC. Possible degradation products were identified with the assistance of LC/MS and GC/MS. The concentration of 2% CPTH on treated rice baits placed in Louisiana rice fields decreased by approximately 55% over 3 days. Several CPTH degradation products were detected in rice field soils, treated with an aqueous solution of CPTH, at concentrations too low for spectral identification. Multiple CPTH degradation products were detected in a laboratory soil metabolism study including the previously unreported compounds, cis- and trans-azo-3-chloro-p-toluidine (azo-CPT). Neither azo compound was detected in soils collected from fields that were treated with CPTH rice baits.

Introduction

Red wing blackbirds (Agelaius phoeniceus) frequently congregate in large spring roosts and cause damage estimated in excess of 3.7 million dollars annually to sprouting rice (1). Damage is particularly severe in Louisiana and tends to be localized and proportional to the size of nearby roost sites (2). As attempts to alleviate damage via habitat manipulation, mechanical and pyrotechnic devices and shooting have been less than successful, recent research efforts have concentrated on the development of an environmentally safe avicide for reducing blackbird populations in localized areas suffering from blackbird-induced rice damage.

In coastal parishes of Louisiana, depredating blackbirds generally roost more than 3 mi from the nearest possible bait site. Glahn and Wilson (3) showed that the flight lines of blackbirds emanating from coastal roosts could be decoyed to simulated rice fields. Studies by West (4) and Knittle et al. (5) showed that, with proper timing and judicious baiting techniques, large roosts of starlings can be selectively reduced by baiting with 3-chloro-p-toluidine hydrochloride (CPTH) baits. Additionally, both laboratory and field studies showed no secondary hazards to non-target species (6, 7).

In conjunction with a study to evaluate the efficacy of CPTH-baited rice fields to reduce localized blackbird populations, we investigated the decrease in CPTH bait concentrations versus time, the migration of CPTH from baits to soil, the decrease in free CPTH soil concentrations versus time, and the formation of CPTH degradation products in soil. We ascertained the decrease in CPTH concentrations in baits and the propensity of CPTH to leach from rice baits to soil by quantifying the CPTH content of the rice bait as well as soil in rice-baited plots at pre-baiting and 1, 3, 6 and 13 days post-baiting. We ascertained the persistence of free CPTH in Louisiana rice fields by applying CPTH directly to soil plots and determining the acetonitrile-extractable CPTH content of soil at the same sampling intervals. Finally, we screened the soil and bait extracts by high-performance liquid chromatography (HPLC) for the presence of CPTH degradation products. Greater quantities of the degradation products were produced by fortifying soils with CPTH at exaggerated levels and incubating under simulated field conditions in an environmental chamber. Extraction of the incubates and co-chromatography with synthesized standards permitted tentative identification of CPTH degradation products. Identification was finalized by gas chromatography/mass spectrometry (GC/MS) and/or liquid chromatography/mass spectrometry (LC/MS).

Experimental Section

Chemicals. All solvents and chemicals were reagent grade unless otherwise specified: acetone, HPLC grade, Fisher Scientific; acetonitrile, optimal grade, Fisher Scientific; benzeno, ACS grade, Fisher Scientific; diethyl ether, J. T. Baker; ethanol, denatured, Fisher Scientific; ethyl acetate, HPLC grade, Fisher Scientific; hexane, optimal grade, Fisher Scientific; CPTH (99.1%), Purina Mills, Inc.; acetyl chloride, Aldrich; 2-chloro-4-nitrotoluene, Chem Service; lithium aluminum hydride, Aldrich; sodium sulfate, ACS grade, 10–60 mesh, Fisher Scientific; deuterated chloroform and deuterated methanol, Aldrich; TLC plates, aluminum backed silica gel, Whatman, 250 μm, 20 × 20 cm, fluorescent at 254 nm; silica gel, Whatman, 70–230 mesh.

Baits. A 2% CPTH-treated rice bait was formulated at the U.S. Department of Agriculture, Animal Damage Control, Pocatello Supply Depot (Pocatello, ID). CPTH concentration was determined by HPLC. The baits were then shipped by truck to the study site in Crowley, LA, and stored in a climate-controlled building.

Quantification of CPTH. All HPLC analyses were conducted on a Hewlett Packard 1090M HPLC system equipped with a diode array detector and a 250 × 4.6 mm Deltabond (Keystone Scientific) 5 μm C-8 column. Injection volumes of 10 μL were used with a water:acetonitrile (70:30) mobile phase at 1 mL/min. CPTH was quantified by comparing absorbance at 241 nm with that of a standard curve generated by analyses of seven calibration standard solutions ranging from 0.02 to 2.0 μg of CPTH/mL of mobile phase. HPLC run times of 14 min were used to quantify the degradation of CPTH. HPLC run times of 35 min were used to analyze soil extracts for the formation of CPTH degradation products.

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The CPTH content of bait samples was quantified following the extraction of approximately 2.0 g of bait with 25 mL of mobile phase in a 50-mL centrifuge tube. The tubes were capped, aligned horizontally, and mechanically shaken at high speed for 10 min, followed by centrifugation at 2000g for 2 min. Extractions were repeated two more times with 10 mL of mobile phase; supernatants were combined and diluted to a final volume of 50.0 mL with mobile phase. Aliquots were filtered through 0.45 µm nylon filters prior to analysis by HPLC.

For analyses of soil samples, approximately 10 g of soil was added to 50-mL centrifuge tubes containing 10.0 mL of acetonitrile. The tubes were capped, aligned horizontally, and mechanically shaken at high speed for 15 min. The soil-containing tubes were then centrifuged at 1000g for 2 min. Aliquots of the supernatant were removed, filtered through 0.45 µm filters, transferred to two 2-mL amber autosampler vials, and capped. CPTH content of the extracts was determined by HPLC. Recoveries were determined by the analysis of quality control (QC) samples containing 0.2, 0.5, and 2.0 µg of CPTH/g of soil. Soil moisture content was determined by calculating mass difference following the drying of 10.00-g aliquots of soil at 110 °C for at least 12 h.

Site Preparation. The rice fields at three different study sites were prepared identically. Fields were divided into 0.3 m × 0.3 m treatment areas. Each treatment area was at least 1 m from any other treatment area. Two application methods, aqueous and seed treatment, were evaluated at each site. For each sampling day, triplicate treatment areas and a control plot were required for each treatment method. Each study site required 24 treatment and eight control areas.

Application Methods. All treatment areas were fortified on the same day by one of two methods. For the rice application method, 30 grains of 2% CPTH-treated rice bait were placed randomly over each 0.09 m² treatment area. The rice bait on each treatment area contained approximately 13.2 mg of CPTH. This application rate is equal to 10 mg of CPTH/mL of water solution was evenly applied with a syringe to control areas. To ensure that the initial quantity of CPTH in contact with the soil was known, an aqueous treatment method was also employed in which 1.00 mL of a 13.2 mg/mL solution was evenly applied with a syringe over each aqueous treatment area. Control areas received no CPTH solution. The field application experiments were completed in the spring of 1994 from March 28 to April 10. The ambient temperatures ranged from 58 to 82 °F with predominately clear skies except for the occurrence of rain on day 12.

Sample Collection. Soil samples were collected one day prior to treatment, several hours after treatment, and 1, 3, 6, and 13 days post-treatment. On each sampling day, a control and three treated samples were collected for each application method; eight samples were collected per sampling day at each site. Each sample was obtained by collecting the top 3 cm deep layer of soil in the desired treatment or control area in a plastic bucket. The soil-containing buckets were covered and mechanically shaken to provide a homogeneous sample of which a 500-g subsample was removed and placed in a 500-mL polyethylene jar. The samples were placed immediately in a freezer, stored for no longer than 4 days, and shipped frozen overnight to our laboratory in Denver. Samples were stored at −26 °C until analyzed.

Storage Stability. Soil samples were stored in our laboratory for 19–33 days prior to analysis. To estimate the loss of CPTH during this storage period, control soils from each study site were combined, homogenized, and fortified at 0, 0.20, 0.50, and 2.00 µg of CPTH/g and stored at identical conditions to the actual field samples. CPTH content of the soils was determined after 0, 8, 15, 26, and 33 days of storage.

Soil Characterization. Representative control soil samples from each study site were sent to the Colorado State University Soil Testing Laboratory for characterization. Conductivity, pH, percent organics, cation exchange capacity, texture analysis, and moisture content were determined for each sample.

Soil Laboratory Incubation. Untreated soils from three sites were combined and mixed, and 10-g aliquots were placed in 60-mL polypropylene centrifuge tubes. The soils were then fortified with CPTH at 300 ppm. Control soils were not fortified with CPTH. The uncovered tubes were incubated in a Revco Model PG-8-1045-A environmental chamber at 25 °C and exposed to a 12:12 h light:dark cycle with an average relative humidity of 65%. The mouths of the uncapped tubes were positioned approximately 0.3 m from a bank of fluorescent and incandescent light bulbs. Approximately 1 mL of deionized water was added to each tube on alternate days. Soil samples were removed on days 0, 1, 3, 6, and 13, extracted, and analyzed by HPLC using the same procedure as was used for the field soil samples.

Mass Spectrometry. HPLC/MS was conducted on a Hewlett Packard (HP) 1090 liquid chromatograph/5989 mass spectrometer equipped with a HP 5990A particle beam interface and operated in the electron impact (EI) ionization mode. Mass spectrometry conditions were as follows: helium nebulizer pressure, 38 psi; ionization potential, 70 eV; desolvation chamber temperature, 400 °C; source temperature, 250 °C. The mass spectrometer was manually tuned using perfluorotributylamine and monitoring ions m/z 69, 219, and 502 in EI mode. Analyses were conducted in the full scan mode (100–300 m/z) and selected ion monitoring mode for the detection of azo-CPT (m/z 125, 153, 278, 280), acetyl-CPT (m/z 106, 141, 183, 185), and 3-hydroxy-p-toluidine (m/z 77, 94, 122, 123). Chromatographic separation was conducted with a 250 cm × 4.6 mm Octyl/HPLC column (Keystone Scientific). A mobile phase of acetonitrile/water (92:8) was used at a flow rate of 0.5 mL/min.

Nuclear Magnetic Resonance Spectroscopy. NMR spectra of the synthesized standards of acetyl-CPT and cis- and trans-azo-CPT were collected on a Bruker-ACE spectrometer, 300 MHz (4H) and 75 MHz (13C).

Synthesis of Standards. Postulated degradation products of CPTH were synthesized for use in identification of actual CPTH degradation products. Acetyl-CPT (N-acetyl-3-chloro-p-toluidine hydrochloride) (Figure 1) was prepared from the freebase of CPTH. The freebase was prepared by differential pH extraction on 489.2 mg of CPTH. A total of 130 mg of the freebase CPTH (0.92 mmol) was taken up in anhydrous diethyl ether, and 1 mL of acetyl chloride (14.2 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 30 min. The reaction was quenched by the addition of distilled water, and the ether was evaporated under vacuo. The white crystals that formed in the aqueous layer were filtered (86.8 mg, 0.47 mmol, 51% yield). Analysis by GC/MS and HPLC indicated a purity of > 99%. Mass spectral data agreed with published data (8). Analysis of 1H NMR (CDCl₃, 7.24 ppm) yielded the following data: H-2, 7.55 ppm (s, 1 p); H-5 and H-6, overlapping, 7.12 and 7.23 ppm (d, 8.4 Hz, d, overlapping solvent peak, 2 p); H-7, 7.29 ppm (s, 3 p); acetyl methyl, 2.14 ppm (s, 3 p).

Azo-CPT (3,3′-Chloro-4,4′-dimethylazobenzene) (Figure 1) was synthesized following the procedure for the reduction of halogenonitroarones with lithium aluminum hydride by
Corbett and Holt (9). A total of 301.2 mg of 2-chloro-4-nitrotoluene (1.76 mmol) was taken up in 30 mL of anhydrous diethyl ether and stirred, with the slow addition of 0.2 g of LiAlH₄. The yellow solution was stirred under nitrogen for 30 min. When distilled water was added to quench the reaction, a white precipitate formed and separated from the clear orange-colored organic layer. The organic layer was decanted and evaporated under vacuo leaving an orange solid. Analysis of the orange solid by GC/MS revealed that the product was a mixture of two major compounds: the azo-CPT compound and that appeared to be the azoxy analogue of the azo-CPT compound.

The azo-CPT compound was separated from the azoxy compound by silica gel flash chromatography with hexane as the eluant. Twenty-two fractions of 8 mL each were collected. The azo-CPT compound eluted in fractions 1–11. With exposure to light during the separation procedure, the trans-azo-CPT isomerized to the corresponding cis isomer. The cis- and trans-azo-CPT compounds were separated using silica gel TLC plates, eluted with 9:1 hexane/acetone. The Rf values for trans- and cis-azo-CPT were 0.81 and 0.58, respectively. Each band was scraped from the TLC plate, and the compounds were recovered from the silica gel by extraction with ethanol.

When analyzed by HPLC, the retention times of the cis- and trans-azo-CPT compounds were 11.1 and 30.1 min, respectively. By GC/MS, the cis- and trans-azo-CPT isomers were indistinguishable. m/z: M⁺ 278 (5), M + 2 280 (3), 153(16), 155 (5), 125 (100), 127 (33), 89 (62).

NMR analysis (CDCl₃, 7.24 ppm). trans-Azo-CPT: H-2, 7.88 ppm (d, 2.1 Hz, 1 p); H-5, 7.36 ppm (d, 8.1 Hz, 1 p); H-6, 7.71 ppm (dd, 8.1, 1.1 Hz, 1 p); H-7, 2.43 ppm (s, 3 p); cis-Azo-CPT: H-2, 6.99 ppm (d, 2.4 Hz, 1 p); H-5, 7.07 ppm (d, 8.1 Hz, 1 p); H-6, 6.52 ppm (dd, 2.4, 8.1 Hz, 1 p); H-7, 2.31 ppm (s, 3 p).

The synthesis of 3-hydroxy-p-toluidine (Figure 1) was performed by the iron-activated reduction of 5-nitro-o-cresol and confirmed by NMR as described by Tawara et al. (10).

**Results and Discussion**

The soil characterization data are presented in Table 1. The soil from site 1 was classified as a silt clay loam while the soil from sites 2 and 3 were characterized as silt loams.

**FIGURE 1. CPTH and degradation products.**

**FIGURE 2. CPTH storage stability curve: ppm CPTH vs days of storage.** Calculated with soil dry weight and recovery corrected. (●) 0.20, (■) 0.52, and (▲) 2.0 ppm.

**TABLE 1. Soil Characterization**

<table>
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<tr>
<th>site</th>
<th>pH</th>
<th>cond.</th>
<th>OM</th>
<th>CEC</th>
<th>sand (g/kg)</th>
<th>silt (g/kg)</th>
<th>clay (g/kg)</th>
<th>texture estimate (g/kg)</th>
<th>water (g/kg)</th>
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<td>1</td>
<td>6.9</td>
<td>0.7</td>
<td>34</td>
<td>188</td>
<td>150</td>
<td>550</td>
<td>300</td>
<td>silty clay loam</td>
<td>173</td>
</tr>
<tr>
<td>2</td>
<td>6.4</td>
<td>0.8</td>
<td>25</td>
<td>142</td>
<td>120</td>
<td>660</td>
<td>220</td>
<td>silt loam</td>
<td>122</td>
</tr>
<tr>
<td>3</td>
<td>6.2</td>
<td>0.6</td>
<td>27</td>
<td>151</td>
<td>170</td>
<td>610</td>
<td>210</td>
<td>silt loam</td>
<td>124</td>
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</tbody>
</table>

All residue data were corrected for QC recoveries generated by analyzing CPTH-fortified soil on the same day of analysis. The mean QC recovery for the three fortification levels over the duration of the study was 43.2 ± 1.1%. In method development experiments, acetonitrile extractions of CPTH-fortified soils gave superior recoveries to other extraction solvents, which included methanol, ethyl acetate, and acetonitrile:water ratios of 50:50 to 100:0. Extractions of CPTH-fortified soil samples with acetonitrile:water (80:20) at pH 3, 6, and 9 also yielded inferior recoveries to acetonitrile.

The storage stability data for the 0.20, 0.52, and 2.0 ppm CPTH-fortified soils were very similar. When compared by analysis of variance (11), the mean storage losses in soil from each study site at the three spiking levels were not significantly different (α = 0.05). Also, the storage-associate losses at each fortification level in soil from different study sites were not significantly different. The mean percent loss ± standard deviation for the three soil types during 33 days of storage were 32 ± 4%, 37 ± 4%, and 33 ± 7% for sites 1, 2, and 3, respectively. The data for each site were combined to generate the storage stability curves (ppm of CPTH vs days of storage) presented in Figure 2. All residue data were also corrected for estimated storage stability losses.

The soil moisture content between sites generally varied by less than 2.5%. On the day of treatment, the mean water content for the soil sampled from the three study sites was 16.8 ± 1.0%. The mean water content decreased to 8.6 ± 1.8% on sampling 6 days post-treatment. Due to rain on day 12, the mean water content increased to 28.7 ± 2.4% in the soil sampled on day 13 post-treatment. Due to this variation...
Concentration (ppm) of free CPTH vs days post-treatment is presented in Figure 3. As the trends in all three sites were similar, the data were plotted as mean residues for all three plots vs days post-treatment. No free CPTH residues were detected in the pre-treatment soil samples. Comparison of the soil residues in the aqueous vs rice bait-treated areas indicated that approximately 40% of the CPTH on the rice seed had migrated to the soil by day 1. Analyses of the CPTH-treated rice bait from the three test sites indicated that the mean half-life of CPTH on the rice seed for the first 6 days of the field test was $2.5 \pm 0.1$ days. These data indicated that the concentration of CPTH on rice seed decreased by approximately 55% by day 3. The rain on day 12 of the experiment apparently contributed to a more rapid decrease of CPTH rice bait residues as the mean half-life for CPTH on the bait was $1.1 \pm 0.02$ days between the days 6 and 13 sampling periods.

CPTH was the only compound detected in the soil extracts from the $10\times$ rice seed bait-treated areas from the field experiment. However, the HPLC chromatograms of extracts of treated soil collected 1–13 days after treatment with the aqueous solution of CPTH indicated the formation of several new compounds. The retention time of CPTH was approximately 4.3 min. Additional chromatographic peaks with retention times of approximately 11.1 and 29.7 min, which were not evident in the day 0 samples, were first detected in the day 1 samples. The relative area of these peaks increased in magnitude through the last sampling date (day 13) when the peak areas of the degradates were approximately 2–10 times that of the parent compound. However, the quantities of degradation products in the soil extracts were insufficient to obtain mass spectral confirmations.

To obtain sufficient quantities of the degradation products for identification by mass spectrometry, control soil samples from the study site were fortified with CPTH and incubated as outlined in the Experimental Section. Comparison of the chromatograms from the analysis of a 13-day fortified soil vs a 13-day control soil again indicated the presence of two CPTH degradates with retention times of 11.1 and 29.7 min. The peak areas of both of these compounds increased over time as the area of the CPTH peak decreased. Also, the area of the 29.7-min peak was approximately five times that of the 11.1-min peak.

As CPTH had a reversed-phase HPLC retention time of 4.3 min, these degradation products were less polar than CPTH. Co-injection of the cis-azo-CPT, trans-azo-CPT, acetyl-CPT, and 3-hydroxy-p-toluidine standards indicated that the peaks with retention times of 11.1 and 29.7 min were due to the presence of cis- and trans-azo-CPT in the fortified soil extracts. The presence of both azo isomers in the soil extract was confirmed by HPLC/MS. Mass spectra of the compounds eluting at these retention times were identical and matched the spectra of the synthesized azo-CPT standard: $m/z$ (relative intensity) 125 (100), 153 (21), 278 (23), 280 (15). The 0.65 ratio of the $m/z = 280$ to the molecular ion $m/z = 278$ is consistent with a dichloro compound. The base ion ($m/z = 125$) corresponds to a fragment formed by the cleavage of the C–N bond and subsequent loss of the azo-chlorotoluene

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**FIGURE 3.** CPTH soil degradation curve: ppm CPTH vs days post-treatment. Calculated with soil dry weight and recovery corrected. (●) Rice, (—) mean rice, (■) aqueous, (—) mean aqueous.

**FIGURE 4.** Mass spectra of azo-CPT.
moiety (m/z = 153). Loss of N₂ via further fragmentation of this azo-chlorotoluene moiety gives a m/z = 125 ion, additionally contributing to the base peak (Figure 4). None of the other standards co-eluted with peaks indicative of degradation products either by HPLC/UV or HPLC/MS. The greater peak area of the later eluting trans isomer in the laboratory incubated soil extracts suggests that the less sterically hindered trans-azo-CPT is favored over the cis isomer.

The presence of azo-CPT was also confirmed by GC/MS. However, it was not possible to resolve both isomers by GC/MS, presumably due to conversion of the cis isomer to the more stable trans isomer at injection port temperatures. The formation of azo metabolites in soils has been reported for a variety of chlorinated anilines. Bartha et al. (12) detected the formation of dichloroazobenzenes from all three isomers of chloroaniline and tetrachloroazobenzene (TCAB) from a variety of dichloroanilines. Kearney and Plimmer (13) detected both cis- and trans-tetrachloroazobenzene in soils fortified with 3,4-dichloroaniline. Azo soil metabolites have also been detected for a variety of pesticides that produced chloro- and dichloroanilines as intermediate metabolites including phenylcarbamates, phenylureas, and acylanilides (14).

It has been reported that the degradation of chloroanilines to azo compounds in soil is mediated by microbial peroxidase activity (12). As peroxidase activity has been reported in a variety of soils (15), the formation of azo compounds from aniline-containing pesticides and environmental contaminants may be widespread. However, it appears that the toxicological significance of these azo compounds is minimal. While most azo compounds are innocuous, a few have been found to be associated with liver tumors in animals (16). Carcinogenicity studies with TCAB, which is structurally similar to azo-CPT, suggest that TCAB does not appear to have the required molecular geometry for carcinogenicity (17). Rats fed TCAB at 4 mg/week for 3 weeks and 10 mg/week for 37 weeks and then finally sacrificed at 60 weeks produced no tumors (18).

To determine the amount of TCAB absorbed and translocated in plants, Still (19) conducted feeding experiments with rice plants in saturated solutions of [14C]TCAB. Of the 14C-TCAB available to the rice plants, 5.6% was absorbed; however, less than 0.2% was found in foliage tissues. Although TCAB was absorbed by rice plants, the results indicated that translocation was minimal. Due to the similarities in structures between TCAB and azo-CPT, these data suggest that if it were available to rice plants, translocation of azo-CPT would be insignificant.

Although azo-CPT was present in the aqueous CPTH-treated soils, no azo-CPT was detected in soils treated with CPTH rice bait at 10× the maximum application rate. The low localized concentration of free CPTH in the rice bait-treated soils may reduce the possibility of polymerization of CPTH to azo-CPT. Therefore under actual field conditions, it appears unlikely that azo-CPT residues would accumulate in soils.

Acetylation of anilines during incubation in soil has been reported for a variety of substrates including 3,4-dichloroaniline (13, 20). Acetyl-CPT was detected on the treated rice bait following exposure to field conditions. The concentration of acetyl-CPT increased on rice sampled from days 1–6 and then decreased from days 6 through 13. The ratio of acetyl-CPT to CPTH increased throughout the study. However, we failed to detect acetyl-CPT in any of the CPTH fortified soil incubates. The failure to detect acetyl-CPT in soils treated with rice bait could be due to cleavage of the acetyl-CPT to CPTH by microbial acylamidases. Such enzymes have been implicated in the degradation of the herbicide propanil (3,4-dichloropropionamide) to 3,4-dichloroaniline (21). Likewise, the substitution of hydroxy moieties for halogens on halogenated benzenes has been documented for numerous compounds under a variety of environmental conditions (22, 23). No soil metabolites coeluted with the 3-hydroxy-p-toluidine standard. The rapid degradation of CPTH under actual field conditions indicates that the propensity for accumulation of the toxicant and the formation of degradation products are minimal.

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Literature Cited

(4) West, R. J. Wildl. Manage. 1968, 21, 637–649.
(6) DeCino, T. J.; Cunningham, D. J.; Schafer, E. W. J. Wildl. Manage. 1966, 30, 249–253.

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