Metabolism of 3,4-Dichloroaniline in Soils

Philip C. Kearney* and Jack R. Plimmer

The recovery and formation of \(^{14}C\)-3,3',4,4'-tetrachloroazobenzene (TCAB) increases as the concentration of \(^{14}C\)-3,4-dichloroaniline increases logarithmically in Hagerstown silty clay loam. A number of products were isolated from soils receiving high applications of propanil (500 to 2000 ppm), and these include 3,3',4,4'-tetrachloroazobenzene (TCAB) (Bartha and Pramer, 1967), 4-(3,4-dichloroanilino)-3,3',4,4'-tetrachloroazobenzene (Linke and Bartha, 1970), and 1,3-bis(3,4-dichlorophenyl)triazene (Plimmer et al., 1968). At lower rates of application (5 to 10 ppm), a major proportion of the 3,4-dichloroaniline is bound to soil particles (Chisaka and Kearney, 1970; Bartha, 1971). The amount of binding may be as high as 80-90% of the added propanil, and involves the intact 3,4-dichloroaniline molecule rather than a metabolite (Bartha, 1971).

Experiments with propanil have usually been conducted at exceptionally high application rates or at low rates of application, typical of agricultural practice, but in no case were attempts made to determine the effect of concentration on the formation of the condensed aniline products. Furthermore, little is known about the nature of aniline decomposition products in soils. Recently Tweedy et al. (1970) isolated and identified the acetylated p-bromoaniline from microbial metabolism of metobromuron [3-(p-chlorophenyl)-1-methoxy-1-methylurea]. They propose that acetylation may serve as a detoxification mechanism by utilizing the aniline formed by metabolism of substituted urea herbicides, thus competing with azobenzene formation.

The 3,4-dichloronitrobenzene (111) was prepared by heating 3,4-dichloroaniline (1.0 g) in ethyl formate (20 ml) for 6 hr under reflux. Excess ethyl formate was removed and the product was recrystallized from benzene:hexane as needles, mp 110°C (lit. mp 110–112°C; Huffman, 1958).

Methods and materials

Ring-labeled \(^{14}C\)-3,4-dichloroaniline (DCA) was prepared from \(^{13}C\) uniformly ring-labeled diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea; specific activity, 1.1 mCi/mmol] by basic hydrolysis (2.5 ml of 1 N NaOH) in 2-methoxyethanol (6.5 ml of redistilled 2-methoxyethanol collected at 124–125°C) after 2 hr of refluxing under nitrogen. The purity of \(^{14}C\)-3,4-dichloroaniline was verified by cochromatography on tlc plates coated with silica gel using a solvent system of benzene:acetone (3:7:1). The isotope was detected on noscreen X-ray film. About 96% of the \(^{14}C\) was recovered as DCA, based on the initial specific activity determined by liquid scintillation.

A concentration series of 1, 10, 100, and 1000 ppm of DCA was established in 100 g of Hagerstown silty clay loam. DCA-\(^{14}C\) (1 \(\mu\)Ci) was added to each soil sample in duplicate. The soils were adjusted to a moisture level equivalent to 70% of field capacity and maintained at 28-30°C for 30 days. Soils were extracted three times with 100 ml of benzene (total 300 ml), the extract (water removed with MgSO\(_4\)) was reduced in volume, and the products were separated by tlc. The \(^{14}C\) spot corresponding to authentic TCAB was removed and the radioactivity determined by liquid scintillation.

A large-scale experiment was initiated with 1 kg of Hagerstown soil containing 1 g of purified DCA plus 5 \(\mu\)Ci of DCA-\(^{14}C\). After 30 days at constant temperature (28-30°C) and moisture content (70% of field capacity), the soil was extracted with 3 l of purified benzene. The soil–benzene slurry was poured into a large filter funnel containing a filter paper covered by a thin layer of diatomaceous earth and dried. The benzene extract was bright orange. The volume of benzene was reduced and radioactivity was measured in the benzene extract before and after extractions with equal volumes (100 ml) of either 1 N HCl or 1 N NaOH. The benzene extract was reduced to 20 ml and added to a column of silica gel (5 cm i.d. \(\times\) 55 cm). The column was eluted successively with 2 l of hexane, 2 l of hexane:benzene (1:1), 2 l of ethyl acetate, and 1 l of ethanol. Ten-milliliter fractions were collected. A small portion was removed from each fraction and chromatographed on tlc using benzene:hexane:ethanol (3:7:1). Tubes containing a single spot were bulked and assayed for radioactivity. Unfortunately, many of the highly-colored fractions appeared to be unstable and, consequently, were discarded if they failed to chromatograph to identical \(R_f\) values after the original separation on tlc. Compounds that yielded a single spot after purification by preparative tlc in two solvent systems were submitted to further analysis by mass and infrared spectrometry.

The 3,4-dichloroformanilide (III) was prepared by heating 3,4-dichloroaniline (1.0 g) in ethyl formate (20 ml) for 6 hr under reflux. Excess ethyl formate was removed and the product was recrystallized from benzene:hexane as needles, mp 110°C (lit. mp 110–112°C; Huffman, 1958).
RESULTS AND DISCUSSION

The amount of total \(^{14}\)C recovered and the amount of TCAB in this recovered \(^{14}\)C increase as the initial concentration of DCA increases logarithmically from 1 to 100 ppm in soil (Table I). As discussed previously (Chisaka and Kearney, 1970; Bartha, 1971), the major reaction of DCA at low concentrations is binding to soil components while a small percentage condenses to form TCAB. However, neither process is a linear function of DCA concentration. From 1 to 100 ppm, concentration reactions favoring TCAB formation are accelerated and increase by a factor of approximately 2 for each tenfold increase in DCA initially added to the soil. It should be emphasized, however, that the amount of soil binding and TCAB formation are strongly influenced by soil type and probably more specifically by the organic matter content of soil (Chisaka and Kearney, 1970). The data suggest that TCAB formation is limited by DCA availability. If all available binding sites are saturated by DCA molecules, the excess DCA may become available for TCAB formation.

Large-Scale Product Study. Approximately 21\% of the radioactivity was recovered in the large-scale experiment involving 1000 ppm of DCA in 1 kg of soil. Extraction of the benzene with 1 N HCl removed only 1.1\% of the total recovered activity, while extraction with 1 N NaOH removed essentially no \(^{14}\)C (<0.01 \%).

Properties of products, isolated from the neutral benzene extract and separated by column and thin-layer chromatography, are shown in Table II. The first two products eluted from the column crystallized in the test tubes in the fraction collector on standing overnight and were subsequently shown to be TCAB or its isomers. Although these compounds exhibited identical properties by mass and infrared spectrometry, they could be separated by tlc in benzene:hexane:ethyl acetate. We suggest that the two orange bands may be due to the cis (I) and trans (II) isomers of TCAB. It is known that light may effect the cis–trans isomerization of azobenzenes (Forbes, 1965). We were unable to confirm that light was responsible for the interconversion of TCAB in this experiment. Other aniline condensation products, including 4-(3,4-dichloroanilino)-3,3',4,4'-tetrachloroazobenzene, 1,3-bis(3,4-dichlorophenyl)triazene, and 3,3',4,4'-tetrachloroazoxybenzene, if formed, could not be detected in the benzene extract. The compound \(m/e\) 161 is unchanged DCA, confirmed by its mass spectrum. It is surprising that this compound occurs in the neutral fraction, but presumably a stronger acid wash is required to extract DCA from the benzene.

A compound of molecular weight 189 was isolated and appeared to be unaltered by the separation procedures. Two chlorine atoms are present in this molecular ion, indicated by the presence of peaks due to ions containing the \(^{35}\)Cl and \(^{37}\)Cl isotopes in the correct intensity ratio. A loss of 28 mass units to \(m/e\) 161 (2 Cl) suggested a close relationship to 3,4-dichloroaniline, which also shows a qualitatively similar fragmentation pathway at lower masses. The loss of ketene during mass spectrometry of N-acetylated amines is well established (Budzikiewicz et al., 1967). The loss of an N-formyl group presumably occurs in an analogous manner by elimination of carbon monoxide. The identity of this compound as 3,4-dichloroformylanilide was confirmed by synthesis and comparison of its physical properties of the material obtained from soil with those of an authentic sample. 3,4-Dichloroformylanilide (III) was prepared by formylation of 3,4-dichloroaniline with acetic formic anhydride (Huffman, 1958). Although Human and Mills (1948) report that sealed tubes or autoclaving may be required for formylation with ethyl formate, we found that this reaction proceeded readily at atmospheric pressure under reflux. Replacement of the ethoxy group takes place readily; however, we found that the analogous acetyl compound could not be obtained by this method and much more vigorous conditions are required (Fieser and Fieser, 1967). The herbicidal activity of 3,4-dichloroformyllanilide was investigated by Huffman and Allen (1960). They observed that the N-acetyl and formyl derivatives were phytotoxic, but were not as effective as propanil.

Formylation represents yet another mechanism by which the amino group of 3,4-dichloroaniline may be modified in soils.

LITERATURE CITED


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Table I. Effect of DCA-\(^{14}\)C Concentration on Extractable Radioactivity and TCAB in Hagerstown Silty Clay Loam

<table>
<thead>
<tr>
<th>Initial DCA-(^{14})C concentration, ppm</th>
<th>(^{14})C recovered, %</th>
<th>TCAB in recovered (^{14})C, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.3</td>
<td>16.7</td>
</tr>
<tr>
<td>10</td>
<td>2.6</td>
<td>37.9</td>
</tr>
<tr>
<td>100</td>
<td>9.0</td>
<td>68.7</td>
</tr>
<tr>
<td>1000</td>
<td>19.0</td>
<td>73.0</td>
</tr>
</tbody>
</table>

Table II. Properties of Some Products Derived from DCA Transformation in Soil (\(^{14}\)C-Labeled)

<table>
<thead>
<tr>
<th>Color</th>
<th>(R_t)</th>
<th>(R_{NH_2})</th>
<th>(R_{OH})</th>
<th>(R_{Cl})</th>
<th>(m/e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>0.89</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>318 (TCAB)</td>
</tr>
<tr>
<td>Yellow</td>
<td>0.74</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>318 (TCAB)</td>
</tr>
<tr>
<td>Yellow</td>
<td>0.29</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>161 (DCA)</td>
</tr>
<tr>
<td>Brown</td>
<td>0.13</td>
<td>-</td>
<td>Weak</td>
<td>+</td>
<td>189</td>
</tr>
<tr>
<td>Brown</td>
<td>0.08</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>171 (no halogen)</td>
</tr>
</tbody>
</table>