# Aerobic and Anaerobic Soil Metabolism of Dicamba

James P. Krueger,† Robert G. Butz,‡ and Douglas J. Cork*,§

Biology Department, Illinois Institute of Technology, Chicago, Illinois 60616, Fitch, Even, Tabin & Flannery, Suite 900, 135 S. LaSalle Street, Chicago, Illinois 60603, and Jellinek, Schwartz, Connolly & Freshman, Inc., Suite 500, 1015 15th Street, N.W., Washington, D.C. 20005

The herbicide dicamba has a half-life of 31 days with a first-order rate constant of 0.0224 $day^{-1}$ in a typical midwestern agricultural soil under aerobic conditions. The half-life of dicamba in the same soil after the soil was made anaerobic at 30 days is 58 days with a first-order rate constant of 0.012 $day^{-1}$. Dicamba is completely mineralized to $CO_2$ under aerobic conditions with 3,6-dichlorosalicylic acid as the only major metabolite. Low levels of 2,5-dihydroxy-3,6-dichlorosalicylic acid were detected. Metabolism under anaerobic conditions is similar to that which occurred in aerobic soil except the rate of dicamba metabolism is reduced under anaerobic conditions.

## INTRODUCTION

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is used as a pre- and postemergent herbicide for control of annual and perennial broad-leafed weeds in corn, small grains, sugarcane, and turf. Dicamba, like other benzoic acid herbicides, has the properties of an auxin-like growth regulator. However, the mechanism of action is still speculative (Frear, 1976). Chemically, dicamba is stable to oxidation and hydrolysis under conventional conditions and is resistant to acid and strong alkali (*Herbicide Handbook*, 1989).

The degradation of dicamba in different soils at different temperatures and moisture levels has been reported (Smith, 1973, 1974, 1984; Smith and Cullimore, 1975; Burnside and Lavy, 1966; Altom and Stritzke, 1973; Stewart and Gaul, 1977). For these studies, the half-life of dicamba varied from 17 to 45 days depending on the soil tested and conditions used; the only metabolites identified by any of these studies were 3,6-dichlorosalicylic acid (3,6-DCSA) and $CO_2$. However, the verification of material balance, including $CO_2$, was absent from many of these studies. No anaerobic studies on the degradation of dicamba were conducted.

Other studies indicate that the degradation of dicamba in aerobic soils and water is biologically mediated (Harger, 1975; Smith 1973, 1974; Smith and Cullimore, 1975; Scifres et al., 1973). Pure cultures of microorganisms that can utilize dicamba as a sole carbon source have been identified from soil and sediment samples (Krueger et al., 1989). Therefore, conditions that promote microbial growth in soil, such as pH, temperature, soil moisture, percent organic matter, and soil composition, generally favor herbicide dissipation (Torstensson, 1988) and have been reported to favor dicamba degradation in soil (Krueger, 1989).

This study describes the dissipation and metabolism of dicamba from a typical midwestern agricultural soil. The half-life and first-order rate constants in aerobic and anaerobic soil were determined. Experiments were conducted under aerobic conditions for 1 year and anaerobically for 9 weeks in the same soil, which was made anaerobic after 30 days of aerobic incubation. The anaerobic study was designed to simulate conditions that may occur when dicamba is leached into anaerobic zones of the soil. The metabolic fate of dicamba in aerobic and anaerobic soil was determined.

* Author to whom correspondence should be addressed.
† Fitch, Even, Tabin & Flannery.
‡ Jellinek, Schwartz, Connolly & Freshman, Inc.
§ Illinois Institute of Technology.

## MATERIALS AND METHODS

**Soil.** The study was conducted with Kenyon loam soil from Cedar Falls, IA. The percentage of sand, silt, clay, organic matter, and organic carbon and bulk density, cation-exchange capacity, pH, and moisture capacity of the Kenyon loam soil were determined according to the methods of Weber (1977).

**Chemicals.** [$^{14}$C]Dicamba (U-*phenyl*-$^{14}$C, mCi/mmol, radiochemical purity >98%) was synthesized by Pathfinder Labs Inc. Authentic reference standards used for the identification of degradation products were synthesized by the Analytical Standard Group of Sandoz Crop Protection Corp. and had a purity of 98% or greater. All other chemicals were of reagent grade or better, and all solvents were of glass-distilled quality.

The [$^{14}$C]dicamba soil treatemnt solution was prepared by mixing 2042 μg of [$^{14}$C]dicamba acid (132.39 μCi) with 6898 μg of analytical reference standard dicamba acid in deionized water. The analytical reference standard dicamba acid was titrated with 0.2 N NaOH to pH 7.0 prior to mixing with the [$^{14}$C]dicamba acid. The resulting specific activity of the treatment solution was 32 875 dpm/μg of dicamba acid.

**Soil Preparation and Treatment.** Kenyon loam soil was maintained in a field moist condition and sieved through 2 mm diameter openings. Field moist soil, equivalent to 2600 g of dry soil, was uniformly wetted with the [$^{14}$C]dicamba treatment solution (prepared as described) to yield a concentration of 2.89 μg of [$^{14}$C]dicamba acid/g of moist soil or 3.44 μg of [$^{14}$C]dicamba acid/g of dry soil. The wetted soil was thoroughly mixed by hand and by mixing on a roller mill for 30 min.

**Study Design.** Treated soil was divided as follows. Two 200-g dry equivalents of soil were weighed into flasks equipped with glass inlet and outlet tubes for the collection of volatile radiocarbon and $^{14}CO_2$ (designated volatiles 1 and volatiles 2). Two 200-g dry equivalents of soil were weighted into 500-mL Erlenmeyer flasks for sampling and quantitation of metabolites (designated metabolite 1 and metabolite 2). Two 200-g dry equivalents of soil were weighed into 500-mL Erlenmeyer flasks and treated with 1000 ppm $HgCl_2$ to prevent microbial growth (designated control 1 and control 2). Two 200-g dry equivalents of soil were weighted into 500-mL Erlenmeyer flasks and used for the anaerobic portion of the study (designated anaerobic 1 and anaerobic 2). The remaining 1000-g dry equivalents of soil were placed in a 4-L Erlenmeyer flask and used for metabolite identification. Water was added to all soils to bring the moisture level to 75% of the 0.33-bar level. Soil moisture was maintained at 75% of the 0.33-bar level throughout the study with addition of deionized water. All soils were incubated at 25 °C in the dark.

0021-8561/91/1439-0995$02.50/0 

**Table I. Properties of Kenyon Loam Soil**

| | | | |
|---|---|---|---|
| % organic C | 2.2 | bulk density, g/cm$^3$ | 1.6 |
| % organic matter (calcd from % organic C) | 3.8 | % sand | 34.0 |
| pH (deionized water) | 6.2 | % silt | 41.0 |
| pH (0.01 M $CaCl_2$) | 6.0 | % clay | 25.0 |
| 75% of 0.33-bar level, g of $H_2O$/100 g of dry soil | 24.4 | textural class | loam |

**Soil Sampling.** Aerobic soils from flasks, designated metabolites 1 and 2 and controls 1 and 2, were sampled for analysis at the following time intervals: 0, 30, 60, 90, 120, 180, 270, and 365 days. A 10-g aliquot was removed from each replicate and frozen at −70 °C until analyzed.

Both 1-L bottles, designated volatiles 1 and 2, were equipped with gas inlet and outlet tubes and were connected to gas washing bottles containing ethylene glycol and 1.5 N KOH to trap any volatile organic compounds and $^{14}CO_2$, respectively. A vacuum was attached to the last trap and adjusted to draw air over the soil at a rate of 10 mL/min. The contents of the $CO_2$ and organic trapping bottles were radioassayed and replaced according to the same schedule used for soil sampling.

To simulate the possibility that a treated soil may become anaerobic after a period of time and that parent compound and/or its metabolites may leach to anaerobic zones of the soil, two flasks of dicamba-treated soil were made anaerobic after 30 days. At 30 days, two flasks of dicamba-treated soil, designated anaerobics 1 and 2 were placed into anaerobic jars (BBL 60627) which were equipped with a double-vented lid. Jars contained anaerobic indicators and were maintained in an anaerobic state by using hydrogen plus carbon dioxide generator envelopes. Anaerobic indicator strips were monitored throughout the study to ensure that anaerobic conditions were maintained throughout the study. At 28 and 58 days after the soil was made anaerobic, jars were purged with nitrogen. Purged nitrogen was passed through ethylene glycol and 1.5 N KOH. At each sampling time, a 10-g soil aliquot was removed and jars were returned to an anaerobic state.

**Sample Analysis.** Aliquots (2.0 mL) of the KOH and ethylene glycol "trapped samples" taken at indicated time intervals from the aerobic and anaerobic experiments were radioassayed in duplicate. All liquid scintillation counting was done by an external quench correction program. Appropriate background counts were subtracted from all samples.

Duplicate 0.5-g samples of soil were mixed with cellulose powder and combusted to $CO_2$ by using a Packard 306 sample oxidizer (Packard Instrument Co., Downers Grove, IL). Standards consisting of 0.5 g of Kenyon loam soil mixed with cellulose powder and a known amount of $^{14}CO_2$ were combusted to determine recovery. Recoveries were used to correct combustion values to 100%.

Ten grams of soil from each sampling time was extracted according to Sandoz Crop Protection GC Residue Method AM-0766. The method involves the base extraction of the soil, followed by an acidified ether extraction of the base extract. Ether extracts and ether-extracted aqueous fractions were radioassayed, and extracted soil was combusted as described. Material balance was determined for each sampling time by converting the total volatilized, $^{14}CO_2$, ether extractable, extracted aqueous, and unextractable fractions into total micrograms (32 875 dpm/μg). The concentration in micrograms per gram of moist soil was obtained by dividing the total micrograms by the amount of sample extracted. The micrograms per gram value was divided by the amount of radiocarbon applied (2.89 μg/g of moist soil) to determine the percent of recovered dicamba substrate equivalents.

Radiocarbon in the ether extractable and extracted aqueous fraction was identified by one-dimensional silica gel thin-layer chromatography. Ether extracts were concentrated and spotted alone and mixed with a standard of dicamba and 3,6-DCSA on duplicate plates. Extracted aqueous fractions were freeze-dried, taken up in methanol, concentrated, and spotted alone and mixed with standard on duplicate plates. Standards (separately and as a mixture) were chromatographed parallel to the sample. One duplicate plate from each sampling time was developed in toluene/acetone/acetic acid (75:20:5 v/v/v) and in ethyl acetate/acetic acid (100:5 v/v) or chloroform/ethanol/acetic acid (85:10:5 v/v/v). Standards were visulized under UV light, and the radiocarbon was located by making autoradiographs of the plates. Radiocarbon on the TLC plate was quantitated by scraping the silica gel from the plate into a liquid scintillation counting vial. Enough methanol was added to completely immerse the silica gel. The vials were sonicated for 0.5 h and radioassayed as described.

Spots that contained radiocarbon corresponding to standards were scraped and extracted with methanol. The methanol extracts were butylated according to Sandoz Crop Protection Residue Method AM-0766 and subjected to GC/MS analysis.

The half-life of dicamba was determined by using the SAS nonlinear regression program (SAS Institute, Cary, NC). Data were fitted to a first-order kinetic model.

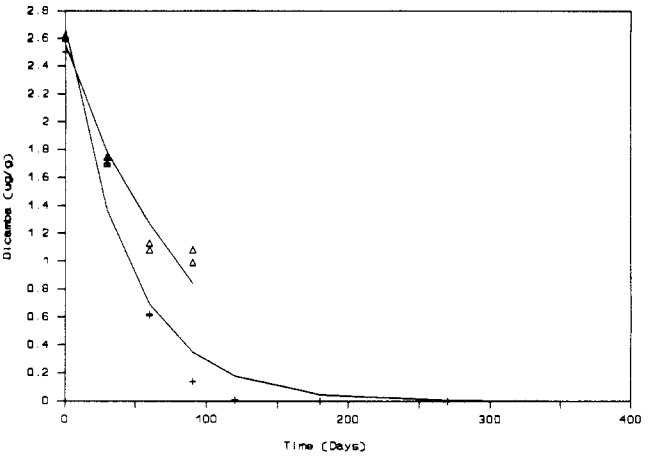


**Figure 1.** Dissipation of dicamba in aerobic Kenyon loam soil (+) and in Kenyon loam soil made anaerobic after 30 days of aerobic incubation (Δ).

**Table II. Total Radiocarbon in [$^{14}$C]Dicamba-Treated Field Moist Aerobic Soil**

| time, days | DPM/g | corrected DPM/g[a] | μg/g | % of applied[b] |
|---|---|---|---|---|
| 0 | 90 072 | 90 732 | 2.76 | 95.5 |
| 30 | 71 234 | 71 723 | 2.18 | 75.4 |
| 60 | 68 567 | 69 035 | 2.10 | 72.7 |
| 90 | 67 692 | 68 153 | 2.07 | 71.6 |
| 120 | 60 863 | 61 269 | 1.86 | 64.4 |
| 180 | 48 959 | 55 052 | 1.67 | 57.8 |
| 270 | 44 395 | 49 913 | 1.52 | 52.6 |
| 365 | 36 787 | 41 346 | 1.26 | 43.6 |
| | | Control Soil[c] | | |
| 0 | 79 678 | 81 596 | 2.48 | 85.8 |
| 30 | 75 800 | 85 276 | 2.59 | 89.6 |
| 60 | 72 180 | 90 441 | 2.75 | 95.2 |
| 90 | 82 919 | 103 897 | 3.16 | 109.3 |
| 120 | 75 215 | 94 245 | 2.66 | 92.0 |
| 180 | 73 983 | 83 229 | 2.53 | 87.5 |
| 270 | 64 984 | 81 424 | 2.48 | 85.8 |
| 365 | 72 853 | 81 956 | 2.49 | 86.2 |

[a] Corrected for combustion recovery of [$^{14}$C]dicamba; combustion recoveries ranged from 88.8% to 99.2%. [b] 100% of 0 time = 2.89 μg/g of moist soil (Table III). [c] Contained 1000 μg/g $HgCl_2$.

## RESULTS AND DISCUSSION

**Dissipation of Dicamba from Aerobic Soil.** The half-life of dicamba in Kenyon loam soil under aerobic conditions was 31 days and the first-order rate constant was 0.0224 day$^{-1}$. The aerobic dissipation curve of dicamba in Kenyon loam soil is shown in Figure 1. The actual data points are indicated, and the curve represents a least-squares fit of the data to a first-order decay equation. The good fit of the data to the computer-

Table III. Total Volatiles and Extraction Characteristics of [$^{14}$C]Dicamba-Treated Aerobic Soil[a]

| day | cum $CO_2$ | cum volatiles | total volatiles[b] | base extractable | ether extractable[c] | aqueous extractable[d] | unextractable | total[e] | % of applied |
|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | 2.83 | 2.74 | 0.00[f] | 0.06 | 2.89 | 100.0 |
| 30 | 0.05 | 0.00 | 0.05 | 2.65 | 2.61 | 0.02 | 0.12 | 2.82 | 97.6 |
| 60 | 0.15 | 0.02 | 0.17 | 1.98 | 1.90 | 0.05 | 0.24 | 2.39 | 82.7 |
| 90 | 0.24 | 0.03 | 0.27 | 1.75 | 1.65 | 0.05 | 0.39 | 2.41 | 83.4 |
| 120 | 0.35 | 0.03 | 0.38 | 1.55 | 1.43 | 0.09 | 0.45 | 2.38 | 82.4 |
| 180 | 0.41 | 0.03 | 0.44 | 1.23 | 1.11 | 0.07 | 0.46 | 2.13 | 73.7 |
| 270 | 0.53 | 0.03 | 0.56 | 0.80 | 0.67 | 0.07 | 0.72 | 2.08 | 72.0 |
| 365 | 0.78 | 0.03 | 0.81 | 1.11 | 0.87 | 0.11 | 0.52 | 2.44 | 84.4 |

[a] Expressed as μg of [$^{14}$C]dicamba equiv/g of moist soil. [b] Total volatiles = $CO_2$ (KOH) + volatiles (ethylene glycol). [c] Extractable after acidification of base extract. [d] Radiocarbon remaining after acidified ether extraction of base extract. [e] Total = total volatiles + base extractable + unextractable. [f] Limit of detection = 0.005 μg of [$^{14}$C]dicamba equiv/g of moist soil or 164 dpm/g. Values less than 0.005 are shown as 0.00.

Table IV. Characterization of Ether and Aqueous Extractable Radiocarbon from [$^{14}$C]Dicamba-Treated Aerobic Soil[a]

| | ether extractable | | | | aqueous extractable | | | |
|---|---|---|---|---|---|---|---|---|
| day | dicamba | 3,6-DCSA | 2,5-diOH | unidentified[b] | dicamba | 3,6-DCSA | 2,5-diOH | unidentified[b] |
| 0 | 2.55 | 0.05 | 0.03 | 0.11 | 0.00[c] | 0.00 | 0.00 | 0.00 |
| 30 | 1.72 | 0.71 | 0.06 | 0.12 | 0.00 | 0.00 | 0.00 | 0.02 |
| 60 | 0.65 | 1.01 | 0.10 | 0.14 | 0.00 | 0.00 | 0.00 | 0.05 |
| 90 | 0.14 | 1.25 | 0.09 | 0.17 | 0.00 | 0.00 | 0.00 | 0.05 |
| 120 | 0.01 | 1.16 | 0.07 | 0.19 | 0.00 | 0.00 | 0.00 | 0.09 |
| 180 | 0.00 | 0.92 | 0.03 | 0.16 | 0.00 | 0.00 | 0.00 | 0.07 |
| 270 | 0.00 | 0.53 | 0.04 | 0.10 | 0.00 | 0.00 | 0.00 | 0.07 |
| 365 | 0.00 | 0.43 | 0.03 | 0.44 | 0.00 | 0.00 | 0.00 | 0.11 |

[a] Expressed as μg of [$^{14}$C]dicamba equiv/g of moist soil. [b] Unidentified does not represent any distinct area of activity on the TLC plate but represents diffuse activity spread over regions of the plate that no standards corresponded to. [c] Limit of detection = 0.005 μg of [$^{14}$C]dicamba equiv/g of moist soil or 164 dpm/g. Values less than 0.005 μg/g are shown as 0.00.

Table V. Total Radiocarbon in [$^{14}$C]Dicamba-Treated Field Moist Soil Made Anaerobic after Aerobic Incubation for 30 Days

| time, days | DPM/g | corrected DPM/g[a] | μg/g | % of applied[b] |
|---|---|---|---|---|
| 0 | 90 072 | 90 732 | 2.76 | 95.5 |
| 30 | 71 234 | 71 723 | 2.18 | 75.4 |
| 58 | 78 015 | 78 982 | 2.40 | 83.0 |
| 93 | 74 612 | 76 477 | 2.33 | 80.6 |

[a] Corrected for combustion recovery of [$^{14}$C]dicamba. [b] 100% of 0 time = 2.89 μg/g of moist soil (Table VI).

generated curve indicates that dicamba dissipation in aerobic Kenyon loam soil follows first-order kinetics. The Kenyon loam soil is typical of a midwestern agricultural soil (soil properties are presented in Table I).

The half-life of dicamba in other aerobic soil studies has been reported to vary from 17 to 45 days depending on the soil tested (Altom and Stritzke, 1973; Smith, 1973, 1974, 1984). Variables such as soil moisture and temperature, percent organic matter, and soil composition influence herbicide degradation. No degradation of dicamba was reported in moist sterile silty clay, heavy clay, or sandy loam (Smith, 1974).

**Reduction of Total Soil Residues in Aerobic Soil.** The results of total radiocarbon assays are shown in Table II. Total radiocarbon decreased steadily to 43.6% of the applied radiocarbon at 365 days. Total radiocarbon in control soil treated with 1000 μg/g $HgCl_2$ did not decrease, suggesting that the aerobic degradation of dicamba is biologically mediated. Extraction and TLC characterization of radiocarbon from 365-day control samples treated with 1000 μg/g $HgCl_2$ were the same at 0 time samples. Considerable evidence in the literature also indicates that the degradation of dicamba in aerobic soil and water is biologically mediated (Harger, 1975; Smith, 1973, 1974; Smith and Cullimore, 1975; Scifres et al., 1973; Krueger et al., 1989).

**Aerobic Soil Metabolism of Dicamba.** Extraction characteristics and total volatiles from [$^{14}$C]dicamba-treated soil are presented in Table III. Total volatiles were mainly in the form of $^{14}CO_2$ (27% of applied) at 365 days with a low percentage of radioactivity in the ethylene glycol trap. The large production of $^{14}CO_2$ is indicative of the rapid and complete mineralization of dicamba.

The major aerobic soil metabolite of dicamba was identified by TLC and confirmed by GC/MS as 3,6-dichlorosalicylic acid (3,6-DCSA). Residues of the 3,6-DCSA increased to a maximum average of 1.25 μg of [$^{14}$C]dicamba equiv/g of moist soil (43.3% of applied) at 90 days and decreased to a level of 0.43 μg of [$^{14}$C]dicamba equiv/g of moist soil (14.9% of applied) at 365 days (Table IV).

TLC and GC/MS analyses identified 2,5-dihydroxy-3,6-dichlorobenzoic acid (2,5-diOH) as a minor soil metabolite. Additionally, GC/MS analysis indicated that the sample extract corresponding to 2,5-diOH on TLC plates also contained approximately an equal amount of 3,6-DCSA and less than 10% 5-hydroxydicamba. The concentration of the fraction corresponding to 2,5-diOH reached a maximum concentration of 0.10 μg of [$^{14}$C]dicamba equiv/g of moist soil (3.5% of applied) at 60 days (Table IV). However, the actual concentration of 2,5-diOH may have been less due to the presence of other metabolites in the same fraction. Significantly, no dichlorophenols were detected in any of the extracts.

Radiocarbon remaining in the aqueous fraction after acidified ether extraction (Table III) was characterized by TLC analysis. Radiocarbon from this fraction remained at the origin of TLC plates and probably represents a number of polar metabolites. However, this fraction reached a maximum of only 0.10 μg of [$^{14}$C]dicamba equiv/g of moist soil at 365 days (Table IV).

The concentration of unextractable radiocarbon increased gradually to 0.52 μg of [$^{14}$C]dicamba equiv/g of moist soil (18.0% of applied) at 365 days (Table III). Base (0.5 N KOH) will release conjugates and adsorbed residues. Therefore, the unextractability of radiocarbon by hot base (65 °C) suggests that the radiocarbon is incorporated into biomass.

Table VI. Total Volatiles and Extraction Characteristics of [$^{14}$C]Dicamba-Treated Aerobic Soil Made Anaerobic at 30 Days[a]

| day | cum $CO_2$ | cum volatiles | total volatiles[b] | base extractable | ether extractable[c] | aqueous extractable[d] | unextractable | total[e] | % of applied |
|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | 2.83 | 2.74 | 0.00 | 0.06 | 2.89 | 100.0 |
| 30 | 0.05 | 0.00 | 0.05 | 2.65 | 2.61 | 0.02 | 0.12 | 2.82 | 97.6 |
| 58 | 0.07 | 0.01 | 0.08 | 2.18 | 2.11 | 0.03 | 0.31 | 2.57 | 88.9 |
| 93 | 0.08 | 0.01 | 0.09 | 2.16 | 2.06 | 0.03 | 0.36 | 2.61 | 90.3 |

[a] Expressed as μg of [$^{14}$C]dicamba equiv/g of moist soil. [b] Total volatiles = $CO_2$ (KOH) + volatiles (ethylene glycol). [c] Extractable after acidification of KOH. [d] Radiocarbon remaining after acidified ether extraction of KOH. [e] Total = total volatiles + base extractable + unextractable.

Table VII. Characterization of Ether and Aqueous Extractable Radiocarbon from [$^{14}$C]Dicamba-Treated Soil Made Anaerobic after Aerobic Incubation for 30 Days[a]

| | ether extractable | | | | aqueous extractable | | | |
|---|---|---|---|---|---|---|---|---|
| day | dicamba | 3,6-DCSA | 2,5-diOH | unidentified[b] | dicamba | 3,6-DCSA | 2,5-diOH | unidentified[b] |
| 0 | 2.55 | 0.05 | 0.03 | 0.11 | 0.00[c] | 0.00 | 0.00 | 0.00 |
| 30 | 1.72 | 0.71 | 0.06 | 0.12 | 0.00 | 0.00 | 0.00 | 0.02 |
| 58 | 1.12 | 0.78 | 0.11 | 0.10 | 0.00 | 0.00 | 0.00 | 0.03 |
| 93 | 1.04 | 0.75 | 0.14 | 0.13 | 0.00 | 0.00 | 0.00 | 0.03 |

[a] Expressed as μg of [$^{14}$C]dicamba equiv/g of moist soil. [b] Unidentified does not represent any distinct area of activity on the TLC plate but represents diffuse activity spread over regions of the plate that no standards corresponded to. [c] Limit of detection = 0.005 μg of [$^{14}$C]dicamba equiv/g of moist soil or 164 dpm/g. Values less than 0.005 μg/g are shown as 0.00.

The total radioactivity recovered and characterized at each time interval (Table III) did accout for most of the radiocarbon applied. Another study done with dicamba in Regina heavy clay resulted in significantly lower material balance in a shorter time interval (Smith, 1974). When mineralization of dicamba was reduced, as in the anaerobic study, material balance was 90.3% of applied at 93 days (Table V). Low material balance is probably due to $^{14}CO_2$ that was not trapped.

**Dissipation of Dicamba from Anaerobic Soil.** The time required for 50% reduction of the dicamba present at 30 days (start of anaerobic conditions) was 58 days and the first-order rate constant was 0.012 day$^{-1}$. The anaerobic dissipation curve of dicamba in Kenyon loam soil is shown in Figure 1.

In studies where soil was flooded to produce anaerobic conditions, the half-life of dicamba was less than 12 weeks (Harger, 1975). Additionally, other studies indicate that chlorinated aromatic herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) were more persistent under anaerobic conditions (Battersby and Wilson, 1989).

**Reduction of Total Soil Residues in Anaerobic Soil.** The results of total radiocarbon assays are shown in Table V. Total radiocarbon did not decrease after the soil was made anaerobic after 30 days of aerobic incubation. The lack of decrease in total radiocarbon indicates that dicamba or its metabolites are not volatilized or mineralized to $CO_2$ under anaerobic conditions.

**Anaerobic Soil Metabolism of Dicamba.** The metabolism of dicamba in aerobic soil made anaerobic after 30 days was similar to its metabolism in aerobic soil. Extraction characteristics and total volatiles from [$^{14}$C]-dicamba-treated soil are presented in Table VI. Total volatiles, which were mainly in the form of $^{14}CO_2$, increased very little to 0.08 μg of [$^{14}$C]dicamba equiv/g of moist soil (2.8% of applied) at 93 days. Studies conducted with several [$^{14}$C]dicamba-treated soils that were flooded to produce anaerobic conditions resulted in $^{14}CO_2$ evolution ranging from 2% to 8% of applied radiocarbon (Harger, 1975).

The major soil metabolite of dicamba-treated anaerobic soil was identified by TLC and confirmed by GC/MS as 3,6-DCSA. Residues of the 3,6-DCSA did not increase significantly after 30 days (start of anaerobic conditions). Residues of the 3,6-DCSA were 0.71 μg of [$^{14}$C]dicamba equiv/g of moist soil at 30 days (24.6% of applied) and increased to a maximum average of 0.78 μg of [$^{14}$C]dicamba equiv/g of moist soil (27.0% of applied) at 58 days (Table VII). Residues of 3,6-DCSA decreased slightly to 0.75 μg of [$^{14}$C]dicamba equiv/g of moist soil (26% of applied) at 93 days.

Experiments designed to analyze the anaerobic metabolism of tricamba (2,3,6-trichlorobenzoic acid) showed dehalogenation at the meta position to yield 2,6-dichlorobenzoate (Horowitz et al., 1983). Suflita et al. (1983) showed that 3,5-dichlorobenzoate was degraded to 3-chlorobenzoate under anaerobic conditions. The dehalogenation of 3,6-DCSA under anaerobic conditions to yield 6-chlorosalicylate might be predicted on the basis of previous studies. However, no evidence of 6-chlorosalicylate formation was seen in the present study.

As in the aerobic dicamba soil study, 2,5-diOH was identified as a minor soil metabolite. A maximum average concentration of 0.14 μg of [$^{14}$C]dicamba equiv/g of moist soil (4.8% of applied) occurred at 93 days.

Characterization of radiocarbon that remained in the aqueous sample after acidified ether extraction once again suggested the presence of a number of polar metabolites. However, the polar radiocarbon remaining at the origin of the TLC plate never went above 0.01 μg of [$^{14}$C]dicamba equiv/g of moist soil.

The concentration of unextractable radiocarbon increased gradually to 0.36 μg of [$^{14}$C]dicamba equiv/g of moist soil (12.5% of applied) at 93 days (Table V). The total radioactivity recovered and characterized at each time interval (Table V) did account for most of the radiocarbon applied and therefore corroborated the validity of the material balance.

Soil conditions favoring degradation by sulfate-reducing methanogenic, denitrifying, or fermentative organisms may result in dicamba degradation. Such conditions were not monitored in the current study. However, in a typical midwestern soil such as Kenyon loam made anaerobic under conditions likely to occur in anaerobic zones of the soil, the dissipation of dicamba and its metabolites was slowed.

## CONCLUSIONS

The half-life of dicamba in aerobic Kenyon loam soil was 31 days with a first-order rate constant of 0.0224 day$^{-1}$. [$^{14}$C]Dicamba was rapidly mineralized, as $^{14}CO_2$ represented a major fraction of the applied radiocarbon (27%)

after 365 days. The lack of degradation in the control ($HgCl_2$) soil indicates that this mineralization is biologically mediated.

Dicamba appears to be first converted to 3,6-DCSA in aerobic Kenyon loam soil by demethylation. The 3,6-DCSA may then be hydroxylated and rapidly metabolized, as only low levels of 2,5-diOH were detected throughout the study. Intermediates that occur after ring opening are apparently unstable and therefore very difficult to isolate. Evolution of $^{14}CO_2$ indicates complete mineralization of dicamba; therefore, unextractable radiocarbon (Table III) presumably is radiocarbon incorporated into biomass. The unextractability of radiocarbon indicates that these residues are not a concern for uptake by rotational crops, exposure to nontarget organisms, or leaching to ground water.

The metabolism of dicamba in soil under anaerobic conditions appears similar to its metabolism in aerobic soil. However, the half-life and first-order rate constant are reduced under anaerobic conditions, 58 and 0.012 $day^{-1}$ anaerobically vs 31 and 0.0224 $day^{-1}$ aerobically, respectively. The short half-life of dicamba in aerobic soil suggests little, if any, dicamba would leach to anaerobic zones of soil. Therefore, the anaerobic degradation of dicamba does not appear to be a major pathway for the dissipation of dicamba in soil for typical agricultural applications.

## LITERATURE CITED


Altom, J. D.; Stritzke, J. F. Degradation of Dicamba, Picloram, and Four Phenoxy Herbicides in Soils. *Weed Sci.* **1973**, *21*, 556–560.

Battersby, N.; Wilson, V. Survey of the Anaerobic Biodegradation Potential of Organic Chemicals in Digesting Sludge. *Appl. Environ. Microbiol.* **1989**, *55*, 433–439.

Burnside, O. C.; Lavy, T. L. Dissipation of Dicamba. *Weed. Sci.* **1966**, *14*, 211–214.

Frear, D. S. The Benzoic Acid Herbicides. In *Herbicides: Chemistry, Degradation and Mode of Action*; Kearney, P. C., Kaufman, D. D., Eds.; Dekker: New York, 1976.

Harger, T. R. Soil Dissipation of Dicamba and VEL-4207. Ph.D. Dissertation, The University of Kentucky at Lexington, 1975.

*Herbicide Handbook*; Weed Science Society of America: Champaign, IL, 1989.

Horowitz, A.; Suflita, J. M.; Tiedge, J. D. Reductive Dehalogenations of Halobenzoates by Anaerobic Lake Sediment Microorganisms. *Appl. Environ. Microbiol.* **1983**, *45*, 1459–1465.

Krueger, J. P. Development of Microbes for Degradation of the Herbicide Dicamba (3,6-Dichloro-2-Methoxybenzoic Acid). Ph.D. Dissertation, Illinois Institute of Technology, Chicago, 1989.

Krueger, J. P.; Butz, R. G.; Atallah, Y. H.; Cork, D. J. Isolation and Identification of Microorganisms for the Degradation of Dicamba. *J. Agric. Food Chem.* **1989**, *37*, 534–538.

Scifres, C. J.; Allen, T. J.; Leinweber, C. L.; Pearson, K. H. Dissipation and Phytotoxicity of Dicamba Residues in Water. *J. Environ. Qual.* **1973**, *2*, 306–309.

Smith, A. E. Transformation of Dicamba in Regina Heavy Clay. *J. Agric. Food Chem.* **1973**, *21*, 708–710.

Smith, A. E. Breakdown of the Herbicide Dicamba and Its Degradation Product 3,6-Dichlorosalicylic Acid in Prairie Soils. *J. Agric. Food Chem.* **1974**, *22*, 601–605.

Smith, A. E. Soil Persistence Studies with Bromoxynil, Propanil and [$^{14}$C]Dicamba in Herbicidal Mixtures. *Weed Res.* **1984**, *24*, 291–295.

Smith, A. E.; Cullimore, P. G. Microbial Degradation of the Herbicide Dicamba in Moist Soils at Different Temperatures. *Weed Res.* **1975**, *15*, 59–62.

Stewart, D. R.; Gaul, S. O. Persistence of 2,4-D, 2,4,5-T and Dicamba in a Dykeland Soil. *Bull. Environ. Contam. Toxicol.* **1977**, *18*, 210–218.

Suflita, J. M.; Robinson, J. A.; Tiedge, J. M. Kinetics of Microbial Dehalogenation of Haloaromatic Substrates in Methanogenic Environments. *Appl. Environ. Microbiol.* **1983**, *45*, 1466–1470.

Torstensson, L. Microbial Decomposition of Herbicides in the Soil. *Outlook Agric.* **1988**, *17*, 120–124.

Weber, J. Soil Properties, Herbicide Sorption, and Model Soil Systems. In *Research Methods in Weed Science*; Truelove, B., Ed.; Auburn Printing: Auburn, 1977.


Received for review July 27, 1990. Accepted December 19, 1990.

**Registry No.** Dicamba, 1918-00-9.