Transformation of Dicamba in Regina Heavy Clay

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The degradation of [^{14}C]dicamba, labeled in the carboxyl group, was studied in moist nonsterile Regina heavy clay at 25 ± 1°. Following soil extraction with 0.1 *M* calcium chloride solution, gas chromatographic and radiochemical analytical procedures were used to monitor the breakdown at weekly intervals. Loss was rapid, with approximately 10% of the applied dicamba being recovered after 5 weeks. Further treatment of the calcium chloride-extracted soils with 1 *N* sodium hydroxide solution recovered small amounts of dicamba (less than 10% of those applied) and increasing quantities of a product identified as 3,6-dichlorosalicylic acid. At the end of 5 weeks, approximately 28% of the dicamba had been transformed into the demethylated compound. Radioactive carbon dioxide was also liberated from the treated soils, indicating that dicamba or the 3,6-dichlorosalicylic acid, or both, underwent decarboxylation.

Dicamba (2-methoxy-3,6-dichlorobenzoic acid) is used as a postemergence herbicide for the selective control of weeds of the buckwheat family in wheat, oats, and barley. It is also applied for the control of broad-leaved weeds in pastures and rangeland grasses.

Investigations have shown that the herbicide can undergo degradation in moist nonsterile soils (Burnside and Lavy, 1966; Corbin and Upchurch, 1967; Donaldson and Foy, 1965; Parker and Hodgson, 1966; Smith, 1973). This breakdown is probably microbial in origin (Parker and Hodgson, 1966; Smith, 1973), since in steam-sterilized soils degradation is inhibited.

The metabolism of dicamba in a number of plant species has been investigated by Broadhurst *et al.* (1966) and Chang and Vanden Born (1971). Both 5-hydroxy dicamba and 3,6-dichlorosalicylic acid were identified in conjugated forms with unidentified plant constituents.

Degradation products of dicamba in soils have not previously been reported and the present study was undertaken to investigate the breakdown of dicamba in moist Regina heavy clay and to identify any degradation products.

MATERIALS AND METHODS

Soil. The Regina heavy clay used in these studies was composed of 70% clay, 25% silt, and 5% sand. The clay also contained 4.2% organic matter and had a pH of 7.5.

Chemicals. [^{14}C]Carboxyl-labeled dicamba (2-methoxy-3,6-dichlorobenzoic-7-^{14}*C* acid) was obtained from the Velsicol Chemical Corp., Chicago, Ill., as were the nonradioactive 5-hydroxy dicamba (5-hydroxy-2-methoxy-3,6-dichlorobenzoic acid) and 3,6-dichlorosalicylic acid. The [^{14}C]dicamba was diluted with nonradioactive herbicide and a methanolic solution was prepared containing 0.5 mg/ml. The specific activity of the dicamba solution was 0.56 μCi/ml.

Degradation Studies. To 42-g samples of the sieved air-dried clay (36 g oven dry) in 300-ml capacity cardboard cartons was added 0.45 ml of the [^{14}C]dicamba solution (225 μg of the herbicide). After thorough mixing, 8.0 ml of distilled water was added to each carton to yield a herbicide concentration of 4.5 ppm based on Regina heavy clay at field capacity moisture (40%). The cartons were loosely capped to permit circulation of air and to reduce water evaporation and incubated in the dark at 25 ± 1°. Distilled water was added every 2 days to maintain the moisture level. Duplicate samples were analyzed after a 24-hr equilibration period and then at weekly intervals for 5 weeks.

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Extraction Procedures. The soils were placed in 125-ml glass-stoppered flasks and 86.0 ml 0.1 *M* aqueous calcium chloride solution was added so that the combined volume of the calcium chloride solution, together with the water present in the soils, was equivalent to 100.0 ml. The flasks were then shaken for 30 min on a wrist-action shaker, after which the contents were filtered under suction and the filtrates were retained. Aliquots (1.0 ml) of each filtrate were examined for radioactivity (see below) and the amount of dicamba extracted was calculated, assuming that all the radioactivity was due to the ^{14}C herbicide.

The soil cakes were washed with an additional 50 ml of fresh calcium chloride solution, the washings being discarded. Following drying at 100°, the soils were ground and thoroughly mixed.

Alkaline Soil Extraction. Samples (10.0 g) of the above dried soils were shaken in a 125-ml capacity glass-stoppered flask with 50.0 ml 1 *N* sodium hydroxide solution for 1 hr on a wrist-action shaker. Following suction filtration, the soil cake was rinsed with a further 25-ml portion of the base and the soil was discarded. The yellow filtrate was acidified with 12 *N* hydrochloric acid and shaken three times with 60-ml volumes of ether. The ether extracts were dried over sodium sulfate and evaporated to dryness using a rotary evaporator, and the residue was dissolved in 10 ml of methanol.

Dicamba Analyses. A 25.0-ml aliquot of each calcium chloride extract (corresponding to 12.5 g of moist clay) was acidified with 1.0 ml 6 *N* hydrochloric acid and extracted four times with 20-ml portions of ether to remove any dicamba present. After drying over sodium sulfate, the ether extracts were evaporated to dryness using a rotary evaporator. The residue was quantitatively transferred, using ether, to a 100-ml capacity glass tube and the ether was evaporated to approximately 5 ml by immersing the tube in a water-bath set at 50°.

The evaporated ether extracts were methylated using 2 ml of a solution of diazomethane in *n*-hexane, according to the procedure described by Rivers *et al.* (1970). Excess diazomethane and ether were volatilized by immersing the tube in a hot water bath, and the volume was adjusted to 25.0 ml with *n*-hexane.

The methylated extracts were diluted with *n*-hexane as necessary. Aliquots of 2 or 3 μl were examined by gas chromatography using an electron-capture detector (Smith, 1973) and the methyl ester of dicamba present was determined. The hexane solution was also checked for radioactivity (see below) and the dicamba content was calculated, presuming that all the activity was due to the [^{14}C]dicamba methyl ester.

Radioactivity. The ^{14}C in the various solutions was measured using a Picker Nuclear Liquimat 200 liquid scintillation spectrometer. The glass vials contained between 0.2 and 1.0 ml of sample, 15.0 ml of scintillation so-

Table I. R_f Values of Compounds Studied

Compound	R_f	
	[a]	[b]
Dicamba	0.66	0.63
5-Hydroxy dicamba	0.29	0.22
3,6-Dichlorosalicylic acid	0.50	0.54

[a] Chloroform–acetic acid (19:1). [b] Benzene–hexane–acetic acid (5:10:2).

Table II. Recovery with Time of [^{14}C]Dicamba from Treated Regina Heavy Clay Incubated at Field Capacity and 25 ± 1°, Using Aqueous Calcium Chloride as Extractant

Time in weeks	Dicamba remaining[a]					
	Gc analysis		Radiochemical analyses			
			I[b]		II[c]	
	μg	%	μg	%	μg	%
0	215	96	216	96	207	92
1	157	70	167	74	158	70
2	152	68	144	64	138	60
3	78	35	90	40	84	37
4	42	19	56	25	52	23
5	17	8	29	13	28	12

[a] Average from duplicate experiments. [b] From direct counting of the aqueous $CaCl_2$ extracts. [c] From the ^{14}C present in the *n*-hexane extracts used for the gas chromatographic analyses.

lution (toluene and 0.4% PPO and 0.1% POPOP), and sufficient methanol (in the case of the aqueous samples) to give a clear homogeneous solution. For the determination of counting efficiencies, [^{14}C]benzene was used as internal standard.

Soil Combustions. Samples (100 mg) of the oven-dried soils were combusted in a platinum holder using a Schöniger combustion flask filled with oxygen. The [^{14}C]carbon dioxide was absorbed in 15.0 ml of a 10% solution of ethanolamine in 2-methoxyethanol and the radioactivity was determined by the counting of suitable aliquots.

Thin-Layer Chromatography. Precoated tlc plates SILG-25 UV 254 were obtained from Macherey-Nagel and Co. (Darmstadt, Germany) and developed with mixtures of either chloroform–acetic acid (19:1) or benzene–hexane–acetic acid (5:10:2). After development, the plates were dried in an oven at 120° for 20 min and sprayed with a solution of bromocresol green (0.04 g) in ethanol (100 ml). With this chromogenic reagent, dicamba, 5-hydroxy dicamba, and 3,6-dichlorosalicylic acid appeared as yellow spots on a blue background.

The R_f values of the compounds studied are shown in Table I.

Radioactive Carbon Dioxide Evolution. This was monitored using the flask method described by Pramer and Bartha (1965). Regina heavy clay samples (50.0 g) at field capacity were treated with 225 μg of [^{14}C]dicamba, with 15.0 ml of 1 *N* sodium hydroxide solution being used to absorb the carbon dioxide evolved. The treated flasks were incubated in the dark at 25 ± 1°, and at weekly intervals the radioactivity in 0.2-ml aliquots of the sodium hydroxide solution from duplicate samples was determined.

RESULTS AND DISCUSSION

The breakdown of dicamba in moist Regina heavy clay, as determined by the gas chromatographic and radiochemical methods, was rapid, with approximately 10% of the applied herbicide remaining after 5 weeks (Table II). Every result represents the average figure for the dicamba recovered from duplicate soil samples at each time interval. In all cases the reproducibility between replicates, whether measured gas chromatographically or radiochemically, was excellent. After each weekly incubation period there was a very close agreement between the amounts of dicamba remaining as detected by the gas chromatographic method and the two radiochemical procedures (Table II). After ether extraction of the calcium chloride soil extracts, no detectable radioactivity remained in the aqueous phase, indicating that all the activity had been partitioned into the organic solvent.

Table III. Percentage of the Applied ^{14}C Remaining on the Clay with Time as Determined by Soil Combustion and Aqueous Sodium Hydroxide Extraction

Time in weeks	% of the applied ^{14}C remaining[a]	
	Combustion	NaOH extraction
1	5	4
2	21	17
3	27	23
4	31	27
5	36	32

[a] Average from duplicate samples, following prior extraction with aqueous calcium chloride.

Within experimental error all the radioactivity recovered from the soils (Table II) using the calcium chloride solution could be accounted for as dicamba by gas chromatographic analysis, which would suggest the absence of significant amounts of any water-soluble ^{14}C degradation products. To confirm the absence of any radioactive degradation products, 25-ml volumes of the calcium chloride extracts obtained from the treated soils incubated for 1, 2, 3, 4, and 5 weeks were acidified and extracted with four 20-ml portions of ether to remove all the activity. The ether extracts were dried over sodium sulfate and evaporated to dryness using the rotary evaporator, and the residues were taken up in 0.5 ml of methanol and applied to the origins of the chromatographic plates. Nonradioactive dicamba, 5-hydroxy dicamba, and 3,6-dichlorosalicylic acid were also applied to the plates as standards. After development and drying, the plates were placed in contact with X-ray film (Kodak RP Royal X-OMAT) for 3 weeks. The autoradiograms indicated a single radioactive compound with each of the chromatographic solvent systems and the R_f value corresponded to that for dicamba (Table I). Cochromatography of the radioactive methanol extract with the pure herbicide, using both solvent mixtures, confirmed the identification.

Duplicate samples of the dried and mixed soils, following the calcium chloride extraction, were combusted and the percentage of the applied radioactivity remaining was calculated. The results (Table III) indicated a steady increase of radioactivity remaining on the soils, with 36% of the original activity being nonextractable with aqueous calcium chloride after 5 weeks. Prior experiments showed that when clay samples treated with [^{14}C]dicamba were combusted, over 90% of the applied ^{14}C could be recovered in the form of [^{14}C]carbon dioxide.

The methanolic extracts derived from the 1 *N* sodium hydroxide treatments of the soils, after the calcium chloride extractions, were examined for radioactivity and 5-ml portions were subjected to thin-layer chromatography using the chloroform and acetic acid developing solvent. From the ^{14}C measurements (Table III), the radioactivity recovered from the soils by the alkaline treatments was in excellent agreement with those determined by the combustion procedure. This would indicate that practically all of the radioactivity not extracted by the calcium chloride solution was subsequently removed by the aqueous sodium hydroxide. Autoradiograms prepared from the developed chromatograms showed the presence of two radioactive products and, at each time interval, they appeared to

Table IV. Radioactivity Recovered from [^{14}C]Dicamba-Treated Soils by Aqueous Calcium Chloride and 1 *N* Sodium Hydroxide with Time

Time in weeks	% of the applied ^{14}C present as: Dicamba, $CaCl_2$ extraction	Dicamba, NaOH extraction	3,6-Dichlorosalicylic acid, NaOH extraction	Total % recovery
1	74	2	3	79
2	64	10	11	85
3	40	8	19	67
4	25	8	23	56
5	13	8	28	49

be the same two compounds. The film darkening due to the ^{14}C compound with the higher R_f value was rather faint and appeared to show a similar intensity after each incubation period, which would suggest that no significant buildup of this compound was occurring in the [^{14}C]dicamba-treated soils. The ^{14}C product with the lower R_f value showed an increasing darkening of the X-ray film with time, indicating a buildup in the treated soils.

The silica gel areas on the chromatograms corresponding to the positions of the two radioactive compounds were removed and exhaustively extracted with methanol, and the methanol was concentrated to small volume. Cochromatography of the various methanol extracts with authentic specimens, using both solvent systems, indicated their identities to be dicamba and 3,6-dichlorosalicylic acid. Additional confirmation for these structural assignments was obtained by methylation of the methanolic extracts with diazomethane, followed by gas chromatographic analysis. In all instances the methyl ester of dicamba was detected. Treatment of 3,6-dichlorosalicylic acid with diazomethane would be expected to methylate both the hydroxyl and carboxy acid groups and would result in the formation of the methyl ester of dicamba. This was confirmed experimentally using the authentic salicylic acid.

Thus, in the soils the dicamba would appear to undergo demethylation to the dichlorosalicylic acid derivative. Separate experiments confirmed that [^{14}C]dicamba underwent no detectable conversion to [^{14}C]3,6-dichlorosalicylic acid on shaking with 1 *N* sodium hydroxide solution in the presence of Regina heavy clay over a 3-hr period.

To determine the relative amounts of dicamba and 3,6-dichlorosalicylic acid recovered from the soils by base, portions of the above methanolic extracts were counted and the activity was determined. Knowing the relative proportions of radioactivity in each of the bands at each weekly interval, and using the ^{14}C combustion data from Table III, the amounts of dicamba and 3,6-dichlorosalicylic acid extracted by the base could be calculated (Table IV). Thus, after 2 weeks, 10% of the applied dicamba was not extracted by the calcium chloride solution, and thereafter this figure remained constant up to 5 weeks, by which time approximately 90% of the original dicamba appeared to have been degraded (Table II). There was a gradual increase of the 3,6-dichlorosalicylic acid with time (Table IV), with 28% of the applied radioactivity being thus accounted for after 5 weeks.

Currently it is not possible to say whether either the 3,6-dichlorosalicylic acid or the base-extractable dicamba were present wholly or in part in chemically combined forms with soil constituents or in a strongly adsorbed physical state. If conjugates were formed, then the basic treatment could have affected hydrolysis during the extraction.

Dicamba is known to undergo negligible adsorption to a variety of soils (Burnside and Lavy, 1966), and Grover (1972) has observed that its adsorption to Regina heavy clay is minimal. Parker and Hodgson (1966) reported that, after 12 months, when residues from 4 and 8 lb/acre field applications of dicamba were no longer detectable by a bean bioassay, the addition of lime caused damage consistent with a residue of approximately 0.01 lb/acre. The present work confirms that small quantities of dicamba can either be strongly adsorbed to or become chemically linked with soil colloids and that the herbicide can be liberated by basic treatment.

As the dicamba used in the present experiments was labeled in the carboxyl group, only those metabolites retaining the originally labeled carbon atom can be radiochemically detected. Table IV summarizes the percentages of the applied radioactivity recovered from the treated soils by the aqueous and basic extractions. There appears (Table IV) to be a gradual loss of radioactivity with time and, at the end of 5 weeks, approximately 50% of that applied remains unaccounted for. This loss could be in the form of [^{14}C]carbon dioxide, formed by a decarboxylation process.

The production of [^{14}C]carbon dioxide from the treated soils indicated a steady evolution, with slightly over 20% of the applied activity being thus liberated within 5 weeks. As these experiments were conducted separately from the degradation studies, it is impossible to conclude whether all the radioactivity unaccounted for in these latter investigations (Table IV) can be attributed to [^{14}C]carbon dioxide losses.

No attempts were made to isolate and identify any of the nonradioactive decarboxylated metabolites; consequently it is not known whether it was the dicamba or the 3,6-dichlorosalicylic acid, or both, which underwent decarboxylation during the soil incubation.

It is considered that both the demethylation and decarboxylation processes were biological rather than chemical in origin, as previous work (Smith, 1973) has shown that in steam-sterilized Regina heavy clay, at the field capacity moisture, dicamba was not significantly degraded over a 4-week period.

5-Hydroxy dicamba has been isolated from wheat and bluegrass plants (Broadhurst *et al.*, 1966) and also from Tartary buckwheat (Chang and Vanden Born, 1971). No trace of this compound was found in any of the present studies.

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