Breakdown of [^{14}C]Dimethylamine in Soils

Allan E. Smith* and Andrew J. Aubin

Research Station, Agriculture Canada, 5000 Wascana Parkway, Box 440, Regina, Saskatchewan S4P 3A2, Canada

The degradation of dimethylamine, used extensively in herbicide formulations, was monitored in three Saskatchewan soils at 85% of field capacity under laboratory conditions at 20 °C using [^{14}C]dimethylamine at rates of 0.5–100 μg/g. In all soils, and at all rates, there was rapid evolution of [^{14}C]carbon dioxide with between 69 and 89% of the applied radioactivity being released after 7 days. The rapid breakdown of dimethylamine in the loamy sand was confirmed by chemical analysis following solvent extraction. In air-dried loamy sand, <1% of the initial radioactivity was released as [^{14}C]carbon dioxide after 7 days, with almost quantitative recovery of the dimethylamine. At [^{14}C]dimethylamine concentrations of 0.5, 10, and 100 μg/g, between 10 and 16% of the applied radioactivity was incorporated into the microbial biomass 7 days after soil treatment.

INTRODUCTION

Dimethylamine formulations of the phenoxyalkanoic acid herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (4-chloro-2-methylphenoxyacetic acid), as well as dicamba (3,6-dichloro-2-methoxybenzoic acid) and other acidic herbicides, are extensively used throughout North America for weed control. From 1987 to 1989, approximately 1 million kilograms (as the acid equivalent) of both 2,4-D and MCPA dimethylamine formulations was applied annually in the three Canadian prairie provinces (Lewis, 1991). From these sources alone, about 400 000 kg of dimethylamine was applied each year to the soils of western Canada.

Adsorption and column leaching studies with 2,4-D and dicamba [^{14}C]dimethylamine salts have indicated that, in soil, dissociation occurs with the [^{14}C]dimethylamine cation becoming strongly adsorbed onto soil colloids (Grover and Smith, 1974). Thus, under field conditions, it is unlikely that dimethylamine will leach from the soil surface. A single laboratory study has reported (Ayanaba et al., 1973) that dimethylamine readily disappeared from moist soils within 4–14 days.

The present study was initiated to provide further information on the fate of dimethylamine in soils by investigating the transformation of [^{14}C]dimethylamine in moist soils to [^{14}C]carbon dioxide and the incorporation of radioactivity into the soil biomass.

MATERIALS AND METHODS

Soils. The three Saskatchewan soils used in this investigation were a Typic Boroll clay and loamy sand from Regina and White City, respectively, and a Udic Boroll clay from Indian Head. The soils were collected in September 1991 from the top 5-cm soil horizon, passed through a 2-mm screen, and stored at 4 ± 1 °C. The studies were conducted during the winter of 1991–1992. Physical characteristics of the soils as determined by the Saskatchewan Soil Testing Laboratory (Saskatoon, SK) are displayed in Table I.

Chemicals. [^{14}C]Dimethylamine, as the hydrochloride salt, was obtained from Sigma Chemical Co. (St. Louis, MO) with a specific activity of 3.45 MBq/mg (1 μCi = 37 kBq) and a radiochemical purity >98%. The [^{14}C]dimethylamine hydrochloride was dissolved in distilled water to yield a solution containing 1.0 MBq/mL and containing 148 μg of dimethylamine/mL. Nonradioactive dimethylamine was obtained as a 40% aqueous solution (Baker Chemical Co., Phillipsburg, NJ) from which stock solutions were prepared, by dilution, containing 1 and 50 mg of dimethylamine/mL of water.

Table I. Composition and Physical Characteristics of Soils

location	soil type	composition, % clay	sand	silt	organic carbon, %	pH	moisture cont at 33 kPa, % w/w
Indian Head	clay	48	22	30	3.7	7.3	38
Regina	clay	62	2	36	3.3	7.6	40
White City	loamy sand	11	80	9	1.6	7.8	15

[^{14}C]Carbon Dioxide Evolution Studies. Soil samples were moistened to 85% of their field capacity moistures (moisture content at 33 kPa) and incubated in the dark for 7 days at 20 ± 1 °C. Water was added (by weighing) as necessary with stirring. Samples (50 g) of all three soils at 85% of their field capacity (FC) were weighed into 175-mL polystyrene foam cartons and placed in 2-L Mason jars fitted with spring-clip lids. The jars were placed in a laboratory incubator in the dark at 20 ± 1 °C for 7 days to allow soil equilibration. Each soil was then treated with [^{14}C]dimethylamine solution (25 μL, 3.7 μg) and sufficient aqueous nonradioactive dimethylamine solution so that treatments were prepared containing 0.5, 10, 50, and 100 μg of dimethylamine/g of moist soil. The soils were stirred with a spatula. There were three replicate treatments for each soil at each amine rate. In each jar was placed a 50-mL beaker inside which was a 20-mL glass vial containing 15 mL of 0.2 M aqueous sodium hydroxide to trap any [^{14}C]carbon dioxide evolved as [^{14}C]-carbonate. After treatment, the jars were reincubated at 20 ± 1 °C. Samples (1 mL) of the sodium hydroxide solution were analyzed for radioactivity after 2, 4 (at which time the caustic vials were replaced with fresh solution), and 7 days. The cumulative amounts of [^{14}C]carbon dioxide released were calculated as a percentage of the total radioactivity applied to the soils. To differentiate between [^{14}C]carbonate and possible volatile ^{14}C products, further 10-mL portions of the trapping solution were treated with aqueous sodium carbonate and barium chloride and the precipitated barium carbonate was removed by centrifugation (Harvey et al., 1985). At all sampling times, the ^{14}C in the trapping vials was attributable to [^{14}C]carbon dioxide. Experiments indicated that <5% of the radioactivity from [^{14}C]-dimethylamine added to 0.2 M sodium hydroxide was precipitated with the barium carbonate.

After the 7-day incubation period, levels of radioactivity from the [^{14}C]dimethylamine incorporated into the soil microbial biomass, except for the 50 μg/g treatment, were determined using a chloroform fumigation–incubation technique (Jenkinson and Powlson, 1976). In this procedure the chloroform disrupts microbial cell membranes, releasing the internal ^{14}C-labeled constituents to microorganisms surviving the fumigation. A portion of these ^{14}C constituents is liberated as a flush of [^{14}C]-carbon dioxide. By measuring this flush and using a mineral-

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Table II. Cumulative Release of [^{14}C]Carbon Dioxide after 2, 4, and 7 Days from Soils Treated with Different Concentrations of [^{14}C]Dimethylamine following Incubation at 20 °C and 85% Field Capacity

dimethylamine concn, μg/g	% of applied radioactivity evolved as [^{14}C]carbon dioxide[a]								
	Indian Head (clay)			Regina (clay)			White City (loamy sand)		
	2 days	4 days	7 days	2 days	4 days	7 days	2 days	4 days	7 days
0.5	81 ± 1	87 ± 1	89 ± 2a	74 ± 1	82 ± 1	84 ± 2a	78 ± 1	84 ± 2	87 ± 2a
10	60 ± 2	67 ± 2	71 ± 3bc	59 ± 3	68 ± 2	71 ± 2b	60 ± 2	71 ± 1	74 ± 1b
50	64 ± 1	71 ± 2	74 ± 2b	60 ± 7	68 ± 4	69 ± 3b	62 ± 3	70 ± 1	74 ± 1b
100	52 ± 3	65 ± 3	69 ± 3c	55 ± 4	67 ± 3	70 ± 3b	43 ± 15	65 ± 5	74 ± 2b

[a] Mean and standard deviation from three replicates. Means within a column followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple-range test.

ization rate factor (K_c) of 0.41, the levels of radioactivity incorporated into the biomass were calculated (Anderson and Domsch, 1978; Biederbeck et al., 1984; Soulas et al., 1984). The method used in the present studies was similar to that previously described for studies with herbicides (Smith, 1988; Smith and Aubin, 1990).

Degradation of [^{14}C]Dimethylamine in White City Loamy Sand. Nine samples (50 g) of White City loamy sand at 85% FC were prepared as described above and fortified with [^{14}C]-dimethylamine solution (25 μL) and sufficient nonradioactive dimethylamine to result in a soil amine concentration of 25 μg/g. Triplicate samples (50 g) of air-dried loamy sand (7% FC) were similarly treated for control purposes. The soils were thoroughly mixed with a spatula. The Mason jars containing the treated soils and sodium hydroxide trapping solution were incubated in the dark at 20 ± 1 °C. After 5 min, 3 days, and 6 days, triplicate samples of the moist soils were removed for analysis. The three air-dried soils were analyzed only after 7 days.

Analyses. [^{14}C]Carbon dioxide evolution was monitored as described above by direct assay of 1.0 mL of the sodium hydroxide solution.

Amounts of radioactivity and dimethylamine in the soil were determined by transferring the soil from each carton to a 250-mL glass-stoppered flask and shaking with 100 mL of aqueous 1 M calcium chloride for 1 h. The flask and contents were allowed to sit overnight before being shaken for a further 1-h period. Following centrifugation at 3500 rmp for 10 min, a 2-mL aliquot was assayed for radioactivity. A further 5-mL portion was analyzed colorimetrically for dimethylamine using a procedure (Patchett et al., 1964) whereby secondary amines are converted to the yellow complex of cupric dithiocarbamate with an absorption peak at 440 nm. In the present study, 5 mL of calcium chloride extract was placed in a 50-mL glass-stoppered tube and 10 mL of benzene added followed by 0.5 mL of copper/ammonia reagent (prepared by dissolving 1.0 g of cupric sulfate in 5 mL of water and diluting to 250 mL with concentrated ammonium hydroxide) and 0.5 mL of carbon disulfide. The tube was stoppered and vigorously shaken by hand for 4 min, and the yellow complex in the organic phase was measured spectrophotometrically at 440 nm. A reagent blank, prepared with 5 mL of 1 M calcium chloride, was used as reference solution. The concentration of extracted dimethylamine was calculated using a standard curve prepared by processing aliquots of dimethylamine in calcium chloride solutions in a similar manner. Prior studies confirmed there were no compounds recovered from untreated soils that would interfere with either the radiochemical or colorimetric analyses. Data from the colorimetric studies were not corrected for recovery efficiency.

After solvent extraction, the soil was collected by vacuum filtration and successively washed with 50 mL of 1 M calcium chloride, 50 mL of water, 50 mL of methanol, and finally 50 mL of acetone. The soils were air-dried overnight at laboratory temperature, and triplicate 1-g samples were then analyzed for residual radioactivity by combustion analysis.

Measurement of Radioactivity. Radioactivity in the solutions was determined using a Packard Tri-Carb 1900 TR liquid scintillation analyzer. Scinti-Verse II (15 mL, Fisher Scientific Co., Fair Lawn, NJ) was the scintillation solution. Counting efficiencies were obtained using a ^{133}Ba external standard. Radioactivity associated with the extracted soils was measured by combustion of 1-g samples in a Harvey biological oxidizer, Model OX500 (R. J. Harvey Instrument Corp., Hillsdale, NJ).

Table III. Percentage of Applied Radioactivity Incorporated into the Biomass following Incubation of Soils with Different Concentrations of [^{14}C]Dimethylamine for 7 Days at 20 °C and 85% Field Capacity

dimethylamine concn, μg/g	% of applied ^{14}C incorporated into biomass[a]		
	Indian Head (clay)	Regina (clay)	White City (loamy sand)
0.5	10.3 ± 0.4a	11.8 ± 0.8a	13.3 ± 0.1a
10	12.8 ± 0.1a	11.8 ± 0.3a	14.7 ± 0.6a
100	12.4 ± 2.5a	13.6 ± 1.4a	15.7 ± 0.7b

[a] Mean and standard deviation from three replicates. Means within a column followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple-range test.

RESULTS AND DISCUSSION

In soil, dimethylamine formulations of dicamba and 2,4-D dissociate (Grover and Smith, 1974), releasing approximately 170 g of amine/kg of formulation. Given that a herbicide application of 1 kg/ha is approximately equivalent to a concentration of 2 μg/g, assuming distribution in the top 5 cm of field soil (Smith, 1988), amounts of dimethylamine in field soils following applications of the two herbicides at such rates would be about 0.3 μg/g. In soils ranging in pH from 5.9 to 7.8 and with organic matter contents between 1.77 and 10.49%, adsorption of [^{14}C]dimethylamine to soil organic matter has been observed with K_d values ranging from 4.5 to 32.6 (Grover and Smith, 1974). Column leaching studies also indicated lack of movement in the soil. Thus, the dimethylamine is likely to remain at the surface of field soils, where much higher concentrations than 0.3 μg/g could be encountered. In the present studies, the breakdown of [^{14}C]dimethylamine was studied at rates up to 100 μg/g.

At all concentrations in soils at 85% FC and at 20 °C, there was very rapid release of the labeled dimethylamine carbon atoms as [^{14}C]carbon dioxide (Table II) with over 40% of the applied ^{14}C being so released after 2 days and 69–89% after 7 days. After 7 days, there were statistically greater amounts of [^{14}C]carbon dioxide released from each soil type treated at the 0.5 μg/g level than from those treated at the 10, 50, and 100 μg/g amine concentrations.

The percentage of the applied radioactivity incorporated into the soil biomass following incubation of the soils for 7 days with 0.5, 10, and 100 μg/g [^{14}C]dimethylamine ranged from 10 to 16% (Table III). Incorporation was independent of amine concentration except possibly for the White City loamy sand incubated with the highest amine concentration (Table III).

The rapid breakdown of [^{14}C]dimethylamine in the three soils confirms similar observations (Ayanaba et al., 1973), where complete loss of 150 and 300 μg/g of the amine was reported after 4 and 14 days, respectively.

Dimethylamine is difficult to extract from treated soils. Ayanaba et al. (1973) used steam distillation to remove dimethylamine from soils, which was then determined

Table IV. Radioactivity and Dimethylamine Recovered with Time from White City Loamy Sand Treated with 25 μg/g [14C]Dimethylamine following Incubation at 20 °C and 85% Field Capacity

14C component	% of applied radioactivity or dimethylamine[a]		
	5 min	3 days	6 days
released as carbon dioxide	<1	70 ± 1	74 ± 1
extracted with 1 M calcium chloride	81 ■ 1 (79 ■ 2)[b]	5 ± 1 (5 ± 4)	3 ± 0 (3 ± 1)[c]
nonextractable, by soil combustion	ND[d]	19 ■ 3	26 ± 1
total 14C recovered	ND	94 ± 3	103 ■ 2

[a] Mean and standard deviation from three replicates. [b] Figures in parentheses represent amounts of dimethylamine recovered, as determined colorimetrically. [c] After 7 days at 20 °C in loamy sand at 7% FC, <1% of the applied radioactivity was released as carbon dioxide and 80 ± 2% of the applied 14C was extractable with calcium chloride. Dimethylamine recovered was 81 ± 3% of that applied. [d] Not determined.

colorimetrically. During the present studies, attempts were made to recover dimethylamine from the soils using solvent extraction with acidic and basic extractants. All recoveries tended to be less than 50% with poor reproducibility. With 1 M calcium chloride, in conjunction with the extended shake, amine recoveries of about 80% with good reproducibility were obtained with the loamy sand. Dimethylamine recoveries from the two clays were less than 60%. A previous study has reported (Grover and Smith, 1974) that adsorption of dimethylamine to a sandy loam with texture analysis and organic content similar to those of the loamy sand used in this study had a much lower K_d value (4.5) than four other soils (9.2–32.6) tested. It was thus assumed that strong adsorption of the dimethylamine cation to the Indian Head and Regina clays was responsible for the poor recoveries. For this reason, studies involving chemical analysis of the amine, following solvent extraction, were conducted only with the loamy sand.

Measurement of dimethylamine and radioactivity in the loamy sand incubated with 25 μg/g [14C]dimethylamine indicated (Table IV) that after 5 min, all of the radioactivity recovered using calcium chloride as extractant was identifiable colorimetrically as dimethylamine. After 3 days of incubation, only 5% of the applied radioactivity was recoverable, and a similar figure was obtained for dimethylamine by the chemical analysis. After 6 days, amounts of 14C and amine recovered were 3% of that applied. During this period there had been considerable release of [14C]carbon dioxide (Table IV). Soil combustion analysis indicated 19% of the applied radioactivity was associated with the soil in a solvent nonextractable form after 3 days and 26% after 6 days. Total accountable radioactivities after 3 and 6 days were therefore 94 and 103% (Table IV), indicating no significant losses of 14C during the study. Of the nonextractable 14C in the soil, at least half was probably due to radioactivity incorporated into the biomass (cf. Table III) and the rest due to 14C incorporated into soil components. In contrast, after 7 days of incubation in air-dried loamy sand, there was no release (<1%) of [14C]carbon dioxide and recoveries of radioactivity and dimethylamine were not significantly different from recoveries from the moist soils after 5 min (Table IV). Thus, this experiment using chemical analysis confirmed the rapid breakdown of dimethylamine in moist loamy sand.

In moist nonsterile soils, trimethylamine is converted by microbial populations to dimethylamine (Ayanaba et al., 1973). Given the accumulation of 14C in the soil biomass in the present studies (Table III), it can be assumed that [14C]dimethylamine, at rates up to 100 μg/g, undergoes similar dealkylation mechanisms with extensive release of [14C]carbon dioxide. It can therefore be concluded that in agricultural soils there will be rapid metabolism of dimethylamine resulting from applications of herbicide formulations with no likelihood of any buildup of such residues.

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