A Metabolic Study of 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (Diuron) Applied to Corn Seedlings

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The metabolism of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) applied to corn seedlings has been studied. Ring-C^{14}–labeled, carbonyl-C^{14}–labeled, and methyl-C^{14}–labeled diuron were synthesized in milligram quantities. Each labeled compound was used in two sets of experiments: application to the leaf, and application to the nutrient solution. Samples of leaf washes, nutrient solutions, corn seedlings, and trapped CO_2 were collected at intervals and analyzed for residues of diuron and its metabolites. The samples were measured by gas chromatography, infrared spectrophotometry, radioactivity, and mass spectrometry. The presence of 3,4-dichloronitrobenzene, 3-(3,4-dichlorophenyl)-1-methylurea, 3-(3,4-dichlorophenyl) urea, and 3,4-dichloroaniline as metabolites in corn seedlings which had been exposed to diuron in the nutrient solution was indicated. A metabolic scheme is proposed to fit the data obtained.

Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], a substituted urea herbicide (Audus, 1964; Crafts, 1961), is used in the treatment of field corn, among other crops. The metabolism of diuron in soil and cotton has been reported by Dalton *et al.* (1965) and the metabolism of a similar compound, 3-(4-chlorophenoxy)-phenyl-1,1-dimethylurea (C-1983), has been reported by Geissbuhler *et al.* (1963). Both groups reported the presence of metabolites and proposed a probable route of metabolism for the parent compound, but did not determine the amounts of each identified metabolite. Although total residues were obtained by the colorimetric method of Pease (1961), this method does not separate the metabolites and will determine only metabolites which can be converted to the chloroaniline structure.

More detailed study of diuron metabolism required a method which could detect and determine diuron and all its metabolites. Gas chromatography offered the best means of carrying out this task. Kirkland (1962) used gas chromatography to determine diuron as 3,4-dichloroaniline, but his procedure was not satisfactory for metabolic studies, and a more specific method had to be developed for the present investigation.

The present work was undertaken to quantitate the residue of diuron and its metabolites in corn seedlings and to follow the fate of diuron when it was applied to the leaves and to the roots of the seedlings. After a gas chromatographic method of analysis had been developed, three forms of C^{14}-labeled diuron were prepared: carbonyl-labeled, methyl-labeled, and ring-labeled. Two sets of experiments were carried out with each labeled compound on corn seedlings: application to the leaf, and application to the nutrient solution.

The seedlings were placed in CO_2-free atmosphere. Appropriate samples were collected at intervals between zero time and 8 days. Leaf washes, nutrient solutions, corn seedlings, and CO_2 traps were analyzed by gas chromatography, infrared spectrophotometry, mass spectrometry, and radioactivity measurements.

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EXPERIMENTAL

Reagents. Acetone, acetonitrile, ethyl acetate, ethyl ether, methylene chloride, petroleum ether, and dioxane (from Burdick and Jackson Laboratories, Inc., Muskegon, Mich.) were distilled in glass.

Washed Celite 545 (Johns-Manville Corp.), 75 grams, and 300 ml. of ethyl acetate were placed in a Waring Blendor jar and blended for 3 minutes. The Celite was filtered through filter paper (S & S 597) in a Büchner funnel with suction, washed with an additional 200 ml. of ethyl acetate, and allowed to air dry.

Animal charcoal (Bone Black), from Matheson Coleman & Bell Corp., was treated in the same manner as Celite 545. Charcoal was mixed with Celite 545, 1 to 10 ratio.

Florisil, washed. About 75 grams of Florisil (60- to 100-mesh) and 500 ml. of water were placed in a 2-liter beaker. The slurry was stirred for 10 minutes, the water decanted off, and the Florisil was transferred to a 12-cm. Büchner funnel (containing a piece of S & S 597 filter paper and under suction) with 200 ml. of acetone. Suction was released and an additional 250 ml. of acetone was allowed to percolate through the Florisil. Suction was reapplied when the acetone stopped dripping and released to allow 250 ml. of ethyl acetate to percolate through the Florisil. Suction was applied once more when the ethyl acetate stopped dripping and the Florisil was air dried and stored in a glass-stoppered bottle.

Anhydrous sodium sulfate, A.R., granulated, washed. About 350 grams of sodium sulfate and 300 ml. of acetone were placed in a Waring Blendor jar and blended at low speed for 3 minutes. The sodium sulfate was filtered through filter paper (S & S 597) in a Büchner funnel with suction. Suction was released and 200 ml. of ethyl acetate were allowed to percolate through the sodium sulfate. Suction was reapplied when dripping ceased. The product was air dried, then heated in a 100° C. oven for 1 hour and stored in a closed container.

Scintillation solution for $Na_2C^{14}O_3$ determination. Seven grams of PPO, 0.3 gram of POPOP, 100 grams of naphthalene, and 50 grams of Cab-O-Sil (thixotropic gel powder) were combined in a beaker and 1000 ml. of dioxane were added.

Scintillation solution for leaf washes, corn seedlings, and nutrient solutions. Same as above but without the Cab-O-Sil.

Apparatus. Gas chromatograph, Barber-Colman Model 5360, with a concentric type electron-capture detector using tritium as the radioactive source.

Gas chromatograph, Packard Instrument Co., with Tri-Carb gas chromatography fraction collector, flame ionization detector.

Tri-Carb liquid scintillation spectrometer, Model 3003, Packard Instrument Co.

Procedure for Metabolism Study. C^{14}-labeled diuron was prepared by a procedure of Todd (1953) as modified by Onley (1966). The authors' modifications of the Todd procedure were designed to produce a theoretical yield of 1 gram of each desired product. Carbonyl-labeled diuron was prepared by the reaction of 3,4-dichloroanilinium salt with C^{14}-labeled phosgene, and the resultant product was allowed to react with dimethylamine. Methyl-labeled diuron was prepared by the reaction of 3,4-dichlorophenyl-isocyanate with C^{14}-labeled dimethylamine. For the preparation of ring-labeled diuron, the first objective was to synthesize ring-labeled 3,4-dichloroaniline. Using labeled *o*-chloroaniline as the starting material, the series of reactions that followed were diazotization, Sandmeyer, nitration, and reduction. The ring-labeled 3,4-dichloroaniline was then carried through the same processes used in the preparation of carbonyl-labeled diuron. In the latter synthesis, unlabeled phosgene was used. The specific activity of carbonyl-labeled diuron was 144 d.p.m. per μg. Methyl-labeled diuron had a specific activity of 26 d.p.m. per μg. Ring-labeled diuron had a specific activity of 36 d.p.m. per μg. These were dissolved in ethanol (2 mg. per ml.).

Corn seeds (certified U.S. 13 Hybrid) were germinated in vermiculite and allowed to grow under the influence of Gro-Lux lamps. After two weeks, the seedlings were removed from the vermiculite and the roots were washed with tap water. Each seedling was placed in an Erlenmeyer flask containing 100 ml. of Hyponex solution (F.W. Bolgiano and Co., Inc., Washington, D. C.) so that its roots were just below the surface of the solution.

Four seedlings were used for each treatment experiment. The seedlings were placed in small, clear, plastic environmental chambers, then set in a greenhouse. CO_2-free air was introduced into the chambers. (Figure 1 shows the experimental design.) After equilibrating for 24 hours, 1.0 mg. of labeled diuron was added to the nutrient solution of each seedling in one set of samples. Twenty micrograms of diuron were placed on a leaf of each seedling in another set of samples. Control samples were carried along with the treated samples. A sample was taken after 0 hour, 1 hour, 5 hours, then 1 day, 3 days, 6 days, and 8 days. Each sampling consisted of nutrient solution, corn seedling, NaOH solution used for trapping any expired CO_2, and—in the experiments on leaf applications—a leaf wash.

Procedure for Collecting and Counting CO_2. The CO_2 was collected in two traps in series; each trap contained 150 ml. of 1*N* NaOH. The contents of both traps were combined when samples were collected. For counting $Na_2C^{14}O_3$, Baron's method (1967) was used. One milliliter of the combined trapping solution was transferred

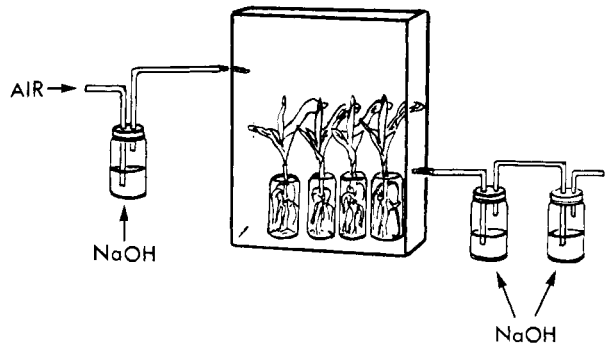

Figure 1. Corn seedlings in CO_2-free environment

to a counting vial containing 2 ml. of methanol. Methanol was used to precipitate the $Na_2C^{14}O_3$. Then, 15 ml. of scintillation solution were added to the vial, mixed well, and counted.

Later, the trapping solutions were concentrated from a volume of 300 ml. to a volume of 100 ml. under a constant stream of CO_2-free air by using a hot plate. The concentrated solutions were analyzed by the same procedure. There was no appreciable difference in the radioactive counts before and after concentration.

General Analytical Procedures. Drying of extracts or washings. Fifty grams of Na_2SO_4 were placed in a filter tube (35 × 200 mm.) plugged with glass wool. The solvent to be dried was percolated through the column and the column was further washed with 50 ml. of solvent.

Evaporation of eluates and washes were all made by a Rinco evaporator.

Gas Chromatographic Analysis. Final solutions were made up to suitable volumes and up to 10 μl. per analysis were injected using the following operational parameters: Column, 6-ft. × 4-mm. I.D. coiled glass column. Packing, 20% Apiezon L on 80- to 100-mesh Gas Chrom Q, preconditioned at 220° C. for 3 days. Column temperature, 210° C.; other temperatures, injection port 210° C.; detector oven 210° C. Electrometer setting, 1×10^{-9} ampere full scale with attenuation of 2. Carrier gas, nitrogen at 50 to 60 ml. per minute. Under these conditions, 10 ng. of diuron gave about 50% full scale deflection. Peak heights and retention times of standards were compared to samples to determine concentration in samples.

C^{14} Analysis of Cleaned-Up Samples. An appropriate aliquot was concentrated in a 22-ml. counting vial, 15 ml. of scintillation solution were added for leaf washes, and the solution was counted.

Procedure for Leaf Wash Analysis. The treated leaf of each seedling was washed with 50 ml. of ethyl acetate. The washes from the four seedlings in the same treatment were combined and dried with the Na_2SO_4 column. After concentrating to 3 to 6 ml. the residue was transferred to suitable volumetric glassware (flask, tube) and aliquots were taken for analysis.

Procedure for Corn Seedlings. After the leaf wash, the roots were rinsed with distilled water and the rinses added to the nutrient solution. Seedlings from the same environmental chamber were combined. Five to 50 grams of corn seedlings were blended for 3 minutes with 10 grams of carbon-Celite mixture and 100 ml. of acetone-water (6 + 4).

A piece of filter paper, S & S 597, was placed in a 12-cm. Büchner funnel and moistened with water. Twenty grams of Celite 545 were packed tightly in the funnel and another piece of filter paper was placed on top of the Celite. The liquid portion of the sample was decanted onto the Büchner, and the plant material was re-extracted with another 100 ml. of solvent. The Blendor cup was rinsed with 40 ml. of solvent.

To isolate diuron and its metabolites, it was necessary to use a long clean-up procedure. The volume of filtrate was adjusted to 230 ml. in a graduated cylinder by adding extraction solvent or evaporating by air jet, then transferred to a 500-ml. separatory funnel. Sixty milliliters of petroleum ether were added and the funnel was shaken vigorously. After the layers had separated, the lower layer was drawn into another separatory funnel. The petroleum ether layer was filtered through Na_2SO_4 into a 500-ml. round-bottomed flask. The aqueous phase was re-extracted with 60 ml. of petroleum ether in the same manner as before; then twice with 60 ml. of petroleum ether–ethyl acetate (1 to 1). The combined filtrates were concentrated to 3 to 6 ml.

The concentrate was transferred to a 125-ml. separatory funnel with 20 ml. of petroleum ether. Forty milliliters of 40% acetonitrile in water (v./v.) were added and the sample was extracted in the usual manner. The aqueous phase was drained into a 500-ml. separatory funnel. The petroleum ether phase was re-extracted three more times and the aqueous phases were combined in the separatory funnel.

Sixty milliliters of ethyl acetate were added to the aqueous extract and this was shaken vigorously for 1 minute. Two hundred milliliters of water were added and shaken gently. After the phases had separated, the aqueous lower layer was drained into another separatory funnel and this was extracted three more times with 60 ml. of ethyl acetate. All ethyl acetate extracts were combined, washed with two 50-ml. volumes of water, dried through a Na_2SO_4 column, and concentrated to 3 to 6 ml.

A chromatographic column (25 × 300 mm. with coarse fritted disk and Teflon stopcock) was prepared containing 5 inches (24 grams) of Florisil topped with $\frac{1}{2}$ inch of Na_2SO_4, or some suitable antisplash material, and washed with 50 ml. of petroleum ether. After discarding the wash, a 500-ml. round-bottomed flask was placed under the column. The concentrate was transferred to the column with several portions of petroleum ether–ethyl acetate (1 + 1) mixture, letting each portion sink into the column before adding the next.

With an elution rate of 5 ml. per minute, the following order of solvent mixture was used to separate diuron from some of its metabolites: the first mixture, 300 ml. of petroleum ether–ethyl acetate (1 to 1), eluted diuron, 3,4-dichloroaniline (3,4-DCA), and 3,4-dichloronitrobenzene (3,4-DCNB). The next mixture, 300 ml. of ethyl acetate, eluted 3-(3,4-dichlorophenyl)-1-methylurea (3,4-DCMU). The last mixture, 250 ml. of 10% acetonitrile in ethyl acetate, eluted 3-(3,4-dichlorophenyl)urea (3,4-DCPU).

Eluates two and three were concentrated to 3 to 6 ml. and analyzed.

For further cleanup of eluate 1, the sample was concentrated to a small volume and transferred to a 10-ml. Erlenmeyer flask with methylene chloride. After carefully concentrating to dryness, using an air jet, 1 ml. of methylene chloride was added to the flask to dissolve contents. The sample was chromatographed on another column containing 10 grams of Florisil topped with an inch of Na_2SO_4. The residue flask was rinsed with several portions of 5% ethyl ether in petroleum ether, and the rinses were added to the column which was then eluted with 150 ml. of the rinsing solvent. This eluted 3,4-DCA and 3,4-DCNB.

Receivers were changed and diuron was eluted from the column with 300 ml. of a mixture of acetonitrile, ethyl ether, and petroleum ether (3:9:88). Each eluate was concentrated to 3 to 6 ml. for analysis.

Procedure for Nutrient Solution. After the seedlings have been removed and the root washings have been added to the nutrient solution, all nutrient solutions from the same environmental chamber were combined and the volume measured. A 100-ml. aliquot was placed in a separatory funnel. This was extracted with 200 ml. of ethyl acetate. The aqueous phase was re-extracted two more times, each time drying the extracts through Na_2SO_4. After concentrating to a small volume, the residue was transferred to 5- or 10-ml. volumetric flasks or graduated tubes with the aid of petroleum ether–ethyl acetate (1 + 1). The volume was adjusted by adding more solvent mixture or by a concentration step, using a stream of dry air. At this stage, the sample is ready for cleanup by Florisil chromatography as described for corn seedlings.

RESULTS AND DISCUSSION

Recoveries. To study recovery of diuron and its metabolites, the compounds were added directly to corn seedlings at the following levels: 0.03 to 10 p.p.m. for diuron, 3,4-DCMU, and 3,4-DCPU; 0.17 to 50 p.p.m. for 3,4-DCA; and 0.05 to 2.0 p.p.m. for 3,4-DCNB. Recoveries at all levels ranged from 74 to 100% as shown in Table I.

The GLC chromatograms of corn seedlings to which diuron had been added at 0.00, 0.025, 0.1, and 1.0 p.p.m. are represented in Figure 2. The injection of 100 mg. of sample resulted in several pronounced peaks as shown in chromatograms *A* and *B*. In chromatogram *C*, diuron at 0.1 p.p.m. was more pronounced than the naturally occurring peaks. In chromatogram *D* where diuron was present at the level of 1.0 p.p.m., virtually all naturally occurring peaks of the corn seedlings were below the sensitivity of detection. The compounds 3,4-DCNB and 3,4-DCA are shown in chromatogram *E*.

Leaf Application. Gas chromatograms of leaf washings sampled at 0 hour and at 3, 6, and 8 days are illustrated in Figure 3. The 3-day sample showed two peaks, the major one being diuron, and the minor one eluting after diuron. This latter peak eluted with the same retention time as 3,4-dichloroaniline. The 6-day sample showed diuron plus two minor peaks, one before and one after diuron. The 8-day sample showed diuron and the peak before diuron. The concentration of this minor peak increased between 6 and 8 days.

Both GLC and radioactive results from the leaf application experiment are presented in Table II. The gas chromatographic results showed a definite trend of decreasing diuron concentration in the leaf wash for all three labeled diurons. This trend became apparent after the first day of exposure.

Table I. Recovery of Diuron and Its Metabolites from Fortified Samples, μg.
By Gas Chromatography

Weight of Sample, G.	Diuron		3,4-DCMU		3,4-DCPU		3,4-DCA		3,4-DCNB	
	Added	Found	Added	Found	Added	Found	Added	Found	Added	Found
5	0.15	0.14	0.15	0.14	0.15	0.13	0.75	0.60		
10	0.25	0.20	0.25	0.25	0.25	0.20	4.0	3.58	0.5	0.48
10	1.0	0.92	0.5	0.49	0.50	0.41	1.7	1.54	20	18.1
10	10	10.0	1.0	0.82	1.0	1.0	3.4	3.01		
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	1.0	0.99	1.0	0.87	1.0	0.97	5.0	4.8	10	8.7
20	20	17.3	20	19.0	20	18.4	100	92.3		
30	150	140	150	146	150	135	750	726		
30	300	290	300	276	300	284	1500	1445		
40	80	70.3	80	68.6	80	75.6	400	322		
40	40	35.5	40	32.1	40	29.4	200	180		
50	5.0	4.2	5.0	3.9	5.0	4.3	25	19.6		
50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
% Recovered, range		80–99		78–100		74–97		78–97		87–96

Diuron = 3-(3,4-dichlorophenyl)-1,1-dimethylurea. 3,4-DCMU = 3-(3,4-dichlorophenyl)-1-methylurea. 3,4-DCPU = 3-(3,4-dichlorophenyl) urea. 3,4-DCA = 3,4-dichloroaniline. 3,4-DCNB = 3,4-dichloronitrobenzene.

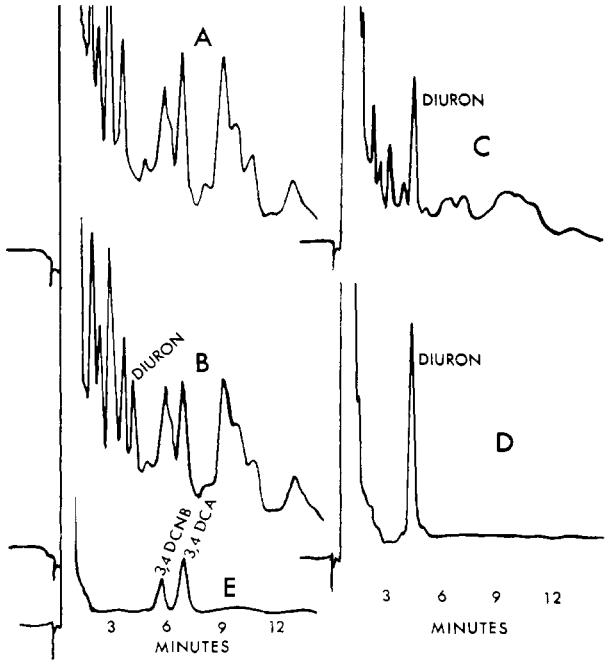

Figure 2. Gas chromatograms

(*A*) control, (*B*) 0.025, (*C*) 0.1, and (*D*) 1.0 p.p.m. of diuron in respective 100-, 100-, 50-, and 10-mg. samples of corn seedlings. Curve *E* represents 0.05 p.p.m. 3,4-dichloronitrobenzene and 0.4 p.p.m. 3,4-dichloroaniline in 100-mg. sample. Flow rate of carrier gas is 60 ml. per minute

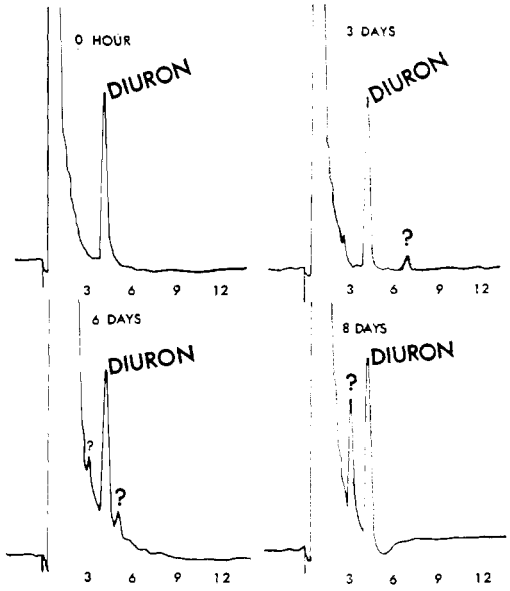

Figure 3. Gas chromatograms of leaf washings at 0 hour, 3, 6, and 8 days

Radioactivity measurements failed to show a similar trend. At the end of 8 days, over 90% of the applied C^{14} was still present, regardless of where the molecule was labeled. Comparison of these results to those obtained by GLC shows that 25 to 60% of the radioactive residue cannot be accounted for by GLC either as diuron or one of its known metabolites. The appearance of the large peak in the GLC chromatogram of the 8-day sample in Figure 3 indicated the presence of a metabolite. Attempts to collect and identify this major peak by infrared were unsuccessful.

When the seedlings were analyzed, only small amounts of diuron were found. No metabolites were detected at the sensitivity level of the method.

The nutrient solutions from these experiments also were analyzed for residues of diuron or its metabolites. None was detected by either GLC or radioactive analysis. As a further check, 500 ml. of a mixture of 6- and 8-day nutrient solution were extracted and the concentrated extract was counted. The total count was the same as the blank.

Since none of the expected metabolites was found in the plants or in the nutrient solutions, either they were not absorbed through the leaf, they did not form, or they were present in amounts too small to be detected. No diuron or metabolites in any form were exuded into the nutrient solution.

Table II. Analyses for Leaf Wash from Leaf Application Experiments[a]

Sample[b]	GLC, μg. of Diuron: Corn seedlings	GLC, μg. of Diuron: Leaf wash	Accountable[c] Diuron: Total, μg.	Accountable[c] Diuron: %	C^{14} Calculated as μg. of Diuron: Corn seedlings	C^{14} Calculated as μg. of Diuron: Leaf wash	Accountable Diuron: Total, μg.	Accountable Diuron: %
				RING LABELED				
0-hour	11.0	66.1	77.1	96.4	11.2	66.4	77.6	97.0
1-hour	10.3	68.7	79.0	98.7	11.4	66.0	77.4	96.8
5-hour	8.2	70.6	78.8	98.6	10.1	62.4	72.5	90.6
1-day	11.7	50.4	62.1	77.6	10.0	62.4	72.4	90.4
3-day	9.8	40.1	49.9	62.4	10.3	63.7	74.0	92.4
6-day	10.4	24.0	34.4	43.1	9.8	63.2	73.0	92.1
8-day	...	...	...	...	9.3	66.0	75.3	94.1
				CARBONYL LABELED				
0-hour	10.6	66.9	77.5	96.8	10.5	66.6	77.1	96.4
1-hour	10.4	66.3	76.7	95.8	10.9	65.2	76.1	95.1
5-hour	9.3	66.3	75.6	94.6	10.0	68.2	78.2	97.6
1-day	11.4	55.9	67.3	84.2	8.2	69.0	77.2	96.3
3-day	11.0	52.2	63.2	79.0	8.9	69.6	78.5	98.2
6-day	11.7	40.9	51.9	64.8	10.3	68.0	78.3	97.8
8-day	10.9	33.7	44.6	55.8	9.9	67.8	77.7	97.0
				METHYL LABELED				
0-hour	8.1	70.2	78.3	97.8	7.7	69.3	77.0	96.2
1-hour	9.6	68.1	77.7	97.0	12.2	60.4	72.6	90.7
5-hour	9.2	63.3	72.5	90.6	11.5	60.6	71.6	89.5
1-day	10.1	62.0	72.1	90.1	9.8	67.2	77.0	96.2
3-day	9.5	62.2	72.7	89.7	10.4	67.8	78.2	97.8
6-day	10.3	53.3	63.6	79.5	9.6	65.5	75.1	93.8
8-day	9.7	50.2	59.9	74.8	11.0	64.8	75.8	94.8

[a] Eighty micrograms of diuron applied to each sample.
[b] All control samples showed no diuron residues.
[c] Values of unknown peaks not included.

Nutrient Solution Application. Analytical results by GLC and C^{14} radioactivity for the seedlings and the nutrient solutions are shown in Table III. This method of application resulted in definite formation of diuron metabolites in both the seedling and nutrient solution. The route of metabolism within the plant seems to be consistent with the routes proposed by Dalton *et al.* (1965) and by Geissbuhler *et al.* (1963). In most instances the relative concentrations found in plants are diuron > 3,4-DCMU > 3,4-DCPU > 3,4-DCA > 3,4-DCNB. The amounts of each compound increased with exposure time indicating an increase in absorption and metabolism. 3,4-DCMU appeared to form rapidly; it was detected by GLC within the plant in 1 hour. Radioactive results were higher than those observed by GLC for all compounds except a few samples of diuron. This indicated the presence of other metabolites containing C^{14} which either were not detectable or could not be resolved as such on the gas chromatograms.

When the 3,4-DCMU fractions of the corn seedlings treated for 3 to 8 days were analyzed, a peak with retention time greater than that of 3,4-DCMU appeared in each eluate (chromatogram *A* of Figure 4). The compound producing this peak could not be positively identified because of an insufficient amount of sample.

The GLC peak believed to be 3,4-DCNB was collected on KBr as described by Giuffrida (1965) and analyzed by infrared spectroscopy (Beckman Model IR 10 with beam condenser). Several samples were combined to provide enough material for infrared analysis. The spectrum of the collected 3,4-DCNB (spectrum *B*) is similar to the spectrum of a standard (spectrum *C*), as shown in Figure 5. A crop blank is represented by spectrum *A*.

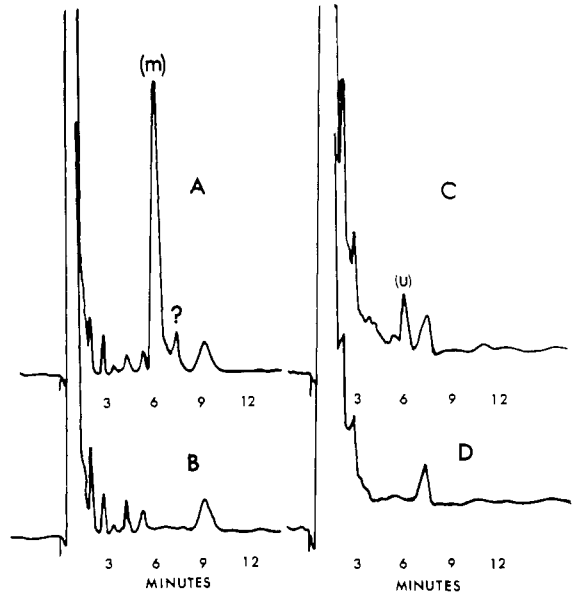

Figure 4. Gas chromatograms

(*A*) 0.7 p.p.m. 3-(3,4-dichlorophenyl)-1-methylurea (m) in 9-mg. sample of corn seedlings, (*B*) 9-mg. control, (*C*) 0.03 p.p.m. 3-(3,4-dichlorophenyl) urea (u) in 45-mg. sample of corn seedlings, and (*D*) 45-mg. control. Flow rate of carrier gas is 50 ml. per minute

Diuron collected from corn seedlings of the nutrient solution application has a spectrum similar to that of a diuron standard not subjected to gas chromatography as illustrated in Figure 6. This indicated that diuron was not degraded in the gas chromatograph under the conditions used.

Table III. Micrograms of Diuron and Metabolites Found by GLC and C^{14} Analyses on Addition of 4 Mg. of Diuron to Nutrient Solutions

	Corn Seedlings										Nutrient Solution										Accountable Diuron			
	Diuron		3,4-DCMU		3,4-DCPU		3,4-DCA		3,4-DCNB		Diuron		3,4-DCMU		3,4-DCPU		3,4-DCA		3,4-DCNB		μg.		%	
Sample	GLC	C^{14}	GLC	C^{14}	GLC	C^{14}	GLC	C^{14}	GLC	C^{14}	GLC	C^{14}	GLC	C^{14}	GLC	C^{14}	GLC	C^{14}	GLC	C^{14}	GLC	C^{14}	GLC	C^{14}
Ring Labeled[a]																								
0-hour	6.1	10.4	0	0	0	0	0	0	0	0	3980	3971	0	0	0	0	0	0	0	0	3986	3981	99.6	99.5
1-hour	33.2	41.6	5.5	11.3	0	0	0	0	0	0	3938	3906	0	0	0	0	0	0	0	0	3977	3959	99.4	99.0
5-hour	60.4	42.2	5.4	15.7	0.14	0.10	0	0	0	0	3912	3805	0	0	0	0	0	0	0	0	3977	3863	99.4	96.5
1-day	58.9	51.2	11.0	38.2	5.5	10.1	0	0	0	0	3881	3711	0	0	0	0	0	0	0	0	3956	3811	98.8	95.2
3-day	70.4	126	28.9	78.8	16.7	15.3	3.6	...	2.2	...	3193	3310	2.8	1.5	0.94	1.7	0	0	0	0	3319	3539	83.4	88.4
6-day	170	287	40.1	133	42.1	59.5	12.5	...	8.1	...	2810	2993	18.1	22.1	4.2	3.3	0	0	0	0	3105	3519	77.5	87.8
Carbonyl Labeled																								
0-hour	13.3	6.9	0	0	0	0	0	0	0	0	3969	3966	0	0	0	0	0	0	0	0	3982	3973	99.6	99.2
1-hour	50.6	25.9	7.1	10.1	0	0.11	0	0	0	0	3912	3900	0	0	0	0	0	0	0	0	3970	3936	99.3	98.4
5-hour	61.0	39.7	10.1	10.8	0.1	0.34	0	0	0	0	3889	3877	0	0	0	0	0	0	0	0	3960	3928	99.0	98.2
1-day	82.2	99.1	22.3	51.3	5.7	18.8	1.5	...	0	0	3744	3770	1.7	0	0	0	0	0	0	0	3857	3944	96.3	98.5
3-day	107	265	39.2	96.0	16.5	88	3.1	...	2.9	...	3301	3431	4.0	2.2	0.61	1.3	0	0	0	0	3474	3890	86.8	97.2
6-day	221	208	52.0	141	57.0	125	10.4	...	5.7	...	3008	3055	24.4	15.5	6.3	2.0	0	0	0	0	3385	3545	84.6	88.5
8-day	324	394	84.6	182	28.4	140	20.9	...	16.0	...	2907	2990	24.8	35.8	7.8	3.4	0	0	0	0	3414	3682	85.2	91.8
Methyl Labeled																								
0-hour	10.1	14.4	0	0	0	0	0	0	0	0	3965	3956	0	0	0	0	0	0	0	0	3975	3970	99.3	99.2
1-hour	26.3	31.3	0.9	0	0	0	0	0	0	0	3932	3940	0	0	0	0	0	0	0	0	3959	3971	98.8	99.2
5-hour	30.8	67.7	4.9	15.4	0.09	...	0	0	0	0	3905	3883	0	0	0	0	0	0	0	0	3941	3966	98.5	99.1
1-day	52.5	80.1	13.4	34.3	1.1	...	0	0	0	0	3808	3800	0	0	0	0	0	0	0	0	3875	3915	96.8	97.8
3-day	131	134	11.6	24.8	6.5	...	1.9	...	0.9	...	3411	3550	1.6	1.1	0.08	...	0	0	0	0	3565	3719	89.2	92.9
6-day	211	208	22.9	59.0	16.7	...	5.6	...	3.2	...	3100	3202	10.0	7.7	1.9	...	0	0	0	0	3371	3504	84.2	87.5
8-day	249	312	16.7	49.2	22.5	...	9.8	...	6.1	...	3051	3193	16.2	19.9	2.7	...	0	0	0	0	3372	3615	84.2	90.2

[a] No 8-day samples were collected for analysis. 3,4-DCA = 3,4-dichloroaniline. Diuron = 3-(3,4-dichlorophenyl)-1,1-dimethylurea. 3,4-DCNB = 3,4-dichloronitrobenzene. 3,4-DCMU = 3-(3,4-dichlorophenyl)-1-methylurea. 3,4-DCPU = 3-(3,4-dichlorophenyl)urea. Corn seedlings controls accompanied all gas chromatographic and radioactive analyses.

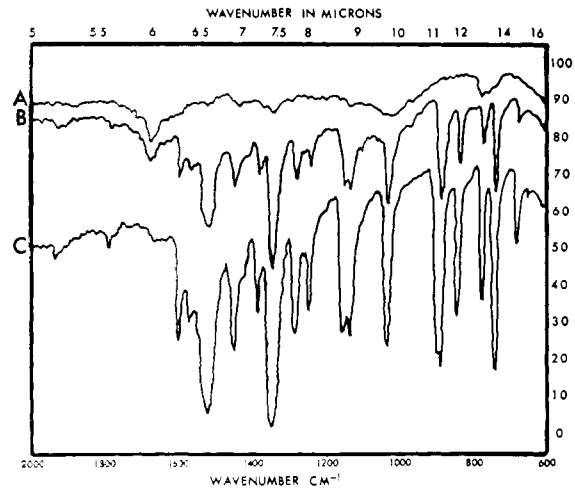

Figure 5. Infrared spectra of material collected gas chromatographically between retention 6.8 to 8 minutes

(*A*) Control corn seedlings, (*B*) treated corn seedlings, and (*C*) 80 μg. of 3,4-dichloronitrobenzene standard

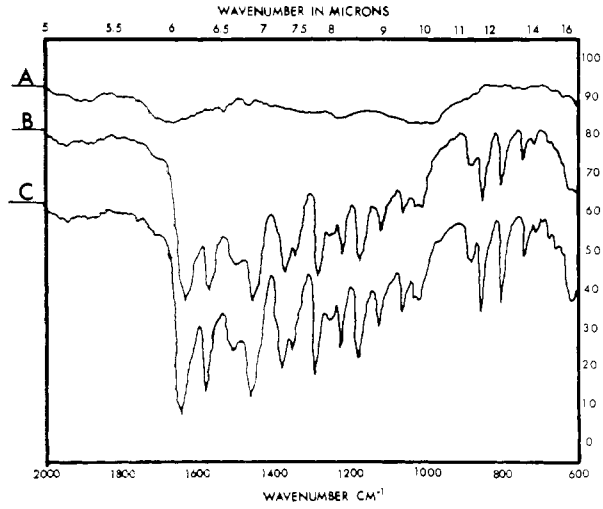

Figure 6. Infrared spectra

(*A*) GLC collected control, (*B*) collected diuron, and (*C*) 100 μg. of diuron standard

Analysis of the nutrient solutions showed that metabolites were present, in contrast to the nutrient solutions for the leaf application experiment. These metabolites did not appear until after the first day of exposure.

Data from Table III showed that more than 95% of the applied diuron could be accounted for by both GLC and C^{14} analyses in the seedling and nutrient solutions for exposures up to one day. From the third day on, the accountable diuron showed a decreasing trend. For carbonyl-labeled and methyl-labeled, the eighth-day samples showed that 85 to 90% of the applied diuron was still present. About 10% of the applied diuron was found within the plants on the eighth day.

Direct C^{14} counts were made of the nutrient solutions for possible water-soluble (nonextractable) metabolites. The total C^{14} counts obtained were similar to the total accountable diuron found (Table III). This shows that the labeled methyl groups and the labeled carbonyl groups were not converted to nonextractable metabolites.

Results showed that the amounts of metabolites were greater within the plants than in the nutrient solutions. This indicates that the metabolites were being formed within the plants and were exuded into the nutrient solution. However, the possibility that the metabolites found in the nutrient solution were actually formed by microbial degradation cannot be ignored completely.

Other Results. Only a small amount of radioactivity was found in the NaOH trapping solutions, indicating that little, if any, of the labeled diuron had been metabolized to $C^{14}O_2$. Other workers have reported a metabolic route involving the oxidation of the *N*-methyl groups to CO_2 to form the reported metabolites. Essentially no $C^{14}O_2$ was observed in this study. It can be concluded that the *N*-methyl groups were not converted to CO_2. Furthermore, the carbonyl group was not converted to CO_2, nor were the carbon atoms in the ring.

The used Florisil columns from the third-, sixth-, and eighth-day samples were checked for the possible retention of C^{14} fragments. None of this Florisil gave a positive test for radioactivity.

Infrared spectra were obtained for 3,4-DCMU, 3,4-DCPU, and 3,4-DCA in addition to 3,4-DCNB and diuron. Their respective KBr disks were later extracted with chloroform and the extract was concentrated to a suitable volume for transferring to the mass spectrometer (Bendix Model 14). Comparisons of the fragmentation patterns for standards with those for compounds isolated from corn seedlings were made. Significant differences in relative peak heights between standards and samples were found in the case of diuron (*m/e* of 72) and 3,4-DCMU (*m/e* of 161). These peaks were higher in the samples than their standards and this could be attributed to the presence of fragments from crop material with the same mass to charge ratios. Sample and standards peaks compared favorably for 3,4-DCPU, 3,4-DCA, and 3,4-DCNB. Regardless of the differences, the necessary fragments were present to show that the samples contained the compounds as predicted.

Based on the above results, a scheme for the metabolism of diuron by the pathways shown in Figure 7 can be postulated. The *N*-demethylation steps could take place in a manner similar to the transmethylation step in the biochemical synthesis of methionine, permitting the removal of the methyl group from diuron without the production of CO_2.

The absence of $C^{14}O_2$ from carbonyl-labeled diuron is puzzling. Normally, the hydrolysis of the C—N bond would be the primary reaction. The products would be isocyanate or carbamic acid, either of which would undergo decarboxylation to CO_2 and free amine. As this was not observed, another explanation must be sought.

The formation of 3,4-dichloronitrobenzene could have resulted from an oxidation process within the corn seedlings or from air oxidation of 3,4-dichloroaniline after it had been extracted from the seedlings. Perhaps both processes were involved. This study has shown, however, that 3,4-DCNB must be considered as part of the residue when analyses are required for diuron and its metabolites.

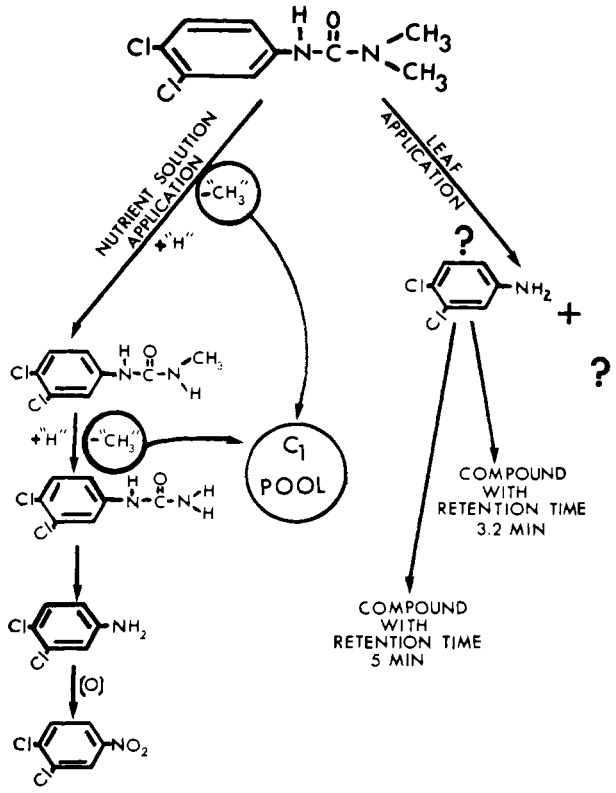

Figure 7. The proposed pathways for metabolism of diuron based on results obtained in this study

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