Investigation of Diuron- and Linuron-Treated Soils for 3,3′,4,4′-Tetrachloroazobenzene

Soil samples from field plots, which had been treated for 12 consecutive years with 2 and 4 pounds per acre of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) and soil samples from a field plot treated with a single application of 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (linuron) at a rate of 2 pounds per acre, showed no detectable 3,3′,4,4′-tetrachloroazobenzene (TCAB). The laboratory results of Bartha and Pramer (1967) were confirmed in that the incubation of 3,4-dichloropropionanilide (propanil), at concentrations of 250 and 500 p.p.m. in soil, yielded substantial residual levels of 3,4-dichloroaniline (DCA) and TCAB after 31 and 42 days. The incubation of 500 p.p.m. of diuron and linuron in soil (equivalent to a field treatment of 500 pounds per acre to a depth of 3 inches), yielded minimal levels of DCA (approximately 1 p.p.m. or less) and no detectable amounts of TCAB, under the same laboratory conditions. Additional studies with DCA in soil up to concentrations of 500 p.p.m. showed, that despite the relatively high initial and residual DCA content, the soil content of TCAB was considerably less than anticipated. Therefore, it is believed that DCA is not the prime precursor for TCAB formed in the soil.

Both 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) and 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (linuron) exhibit favorable patterns of disappearance in a variety of soils (Hill *et al.*, 1955; Hill *et al.*, 1962; Dalton *et al.*, 1966; Lode, 1967). The soil degradation products of diuron isolated by Dalton *et al.* (1966) include 3-(3,4-dichlorophenyl)-1-methylurea, 1-(3,4-dichlorophenyl)urea, and 3,4-dichloroaniline (DCA). Cotton roots grown in solution culture containing labeled diuron generate the same metabolites (Smith and Sheets, 1967). Nash (1968) identified the presence of diuron and 3-(3,4-dichlorophenyl)-1-methylurea in roots and shoots of oat-seedlings grown in diuron-treated Lakeland sandy loam soil. Geissbühler *et al.* (1963) identified analogous metabolites in their microbiological and plant studies with 3-(*p*-chlorophenoxyphenyl)-1,1-dimethylurea (chloroxuron).

Bartha and Pramer (1967) and Bartha (1968) found that herbicide, 3,4-dichloropropionanilide (propanil), when incubated in soil under laboratory conditions at a concentration of 500 p.p.m., yielded DCA and 3,3′,4,4′-tetrachloroazobenzene (TCAB). They postulated that DCA condenses with itself to form TCAB, or that DCA, in part, may be transformed to 3,4-dichloronitrosobenzene which then condenses with DCA to form TCAB. In further laboratory studies, Bartha *et al.* (1968) detected the corresponding dichloro- and tetrachloroazobenzenes in soils treated with 1000 p.p.m. of all monochloroanilines and some of the dichloroanilines. On this basis, these workers postulated that the phenylurea and phenylcarbamate herbicides may give rise to TCAB or other azobenzenes in the soil, since the corresponding anilines are degradation products of these herbicides (Kearney and Kaufman, 1967). Recently, Kearney *et al.* (1969) reported the absence of 3,3′-dichloroazobenzene in three different soils 60 days after treatment with isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) at a concentration of 1000 p.p.m.

The present studies were initiated to determine whether linuron and/or diuron yield TCAB in the soil under practical field conditions and under laboratory conditions as used by Bartha and Pramer (1967).

APPARATUS AND REAGENTS

The MT-220 gas chromatograph (Micro-Tek Instruments, Inc., Baton Rouge, La.) was equipped with a Dohrmann microcoulometer, T-300S titration cell, and S-100 sample inlet/combustion unit. The chromatographic column was 5% General Electric XE-60 silicone gum plus 0.2% Epon Resin 1001 on 60- to 80-mesh Gas Chrom Q (Applied Science Lab., Inc., State College, Pa.), 4 feet, glass, $^1/_4$-inch O.D., $^3/_{16}$-inch I.D. The Beckman Model B spectrophotometer was used in the colorimetric determination of DCA. Diuron and linuron were standard reference materials, available from the Industrial and Biochemicals Department, Agrichemical Sales Division, E. I. du Pont de Nemours and Co., Wilmington, Del. The 3,4-dichloroaniline used in this study had been recrystallized from 70% aqueous ethanol. The 3,3′,4,4′-tetrachloroazobenzene used as a standard was synthesized by the procedure of Gaudry and Keirsted (1949), and had a M.P. of 156–8° C. The *n*-hexane was a Distilled-in Glass solvent, Burdick & Jackson Laboratories, Inc., Muskegon, Mich.

EXPERIMENTAL

Gas Chromatographic Calibration. The gas chromatograph was equilibrated as follows—vaporizer temperature, 230° C.; transfer temperature, 250° C.; furnace temperature, 850° C.; column temperature, 200° C.; carrier flow, helium 100 cc. per minute; purge flow, helium 50 cc. per minute; oxygen flow, 50 cc. per minute.

The column was conditioned by maintaining its temperature at 225° C. for at least 48 hours. A calibration curve was prepared by chromatographing appropriate aliquots of a standard solution containing 10 μg. per ml. of TCAB in hexane, and plotting peak heights *vs.* micrograms of TCAB injected. Peak height was used because of the base line elevation obtained at the high chromatographic temperatures of this method. This base line shift nullifies the advantages of an electromechanical integrator for determining peak area. For TCAB calibration, the column temperature was set at 140° C., with the coulometer sensitivity set at 90 ohms. Appropriate volumes of TCAB standard solutions were injected to deliver 0.1 to 0.8 μg. of TCAB. Two minutes after injection, the column was programmed at 5° C. per minute to an upper temperature limit of 190° C., at which point it was maintained for approximately 10 minutes to recondition the column. The retention time for TCAB from the start of programming was about 9 minutes. A typical gas chromatographic scan of a standard TCAB solution is shown in Figure 1.

Exploratory evaluations indicated that dichloroaniline in the soil extracts can also be analyzed by a gas chromatographic technique, using the same column. The temperature range for this analysis was 90° to 190° C. and the temperature was programmed at 7.5° C. per minute. Preliminary data have

indicated good agreement between the values obtained by this procedure and the accepted colorimetric procedure used for DCA. For this study, however, the DCA data were obtained using the accepted colorimetric procedure.

The response to both DCA and TCAB was linear throughout the ranges studied.

Laboratory Soil Treatments. Fifty-gram samples (air-dry basis) of Keyport-type silt loam soil, whose pH had been adjusted to 6.5 to 6.8 with $CaCO_3$, were introduced into a series of flasks. Diuron, linuron, propanil, and DCA were added to the soils as acetone solutions in sufficient quantities to provide the desired final concentrations. The solvent was permitted to evaporate overnight from the soil. The flasks were rotated gently to ensure good mixing and distribution of chemical in the soil. Water was added to the soil to elevate the water content to 50% of the total water-holding capacity of the soil. To ensure sufficient viable soil microorganisms, a supernatant from a water suspension of a garden soil was used as the water to elevate the soil moisture content. The flasks were plugged with cotton and incubated at 27° C. for the entire experimental period. Sufficient water was added to all flasks every 3 or 4 days to maintain the water content of the soil.

Field Soil Treatments. The diuron field plots from which soil samples were taken had been treated with 0, 2, and 4 pounds of diuron per acre for 12 consecutive years. The samples were taken approximately a year after the last treatment. The linuron field plots, which were sampled 2 months after treatment, had received a single application of 0 and 2 pounds of linuron per acre. In all the above test plots, the soil was a Keyport-type silt loam.

Extraction. The procedure used for the isolation of DCA and TCAB from soil was patterned after that of Bartha and Pramer (1967). The 50 grams of check or treated soil was extracted three times with 100-ml. volumes of acetone. The acetone extracts were combined and the extract volume reduced to 50 to 60 ml. Approximately 150 ml. of water was then added and the pH was adjusted to 11.0 to 11.5 with NaOH. Sufficient NaCl (approximately 65 grams) was added to saturate the system, which then was extracted three times with 50-ml. portions of *n*-hexane. The hexane extracts were combined and the volume was reduced to a suitable size (1.0 to 5.0 ml.) for DCA and TCAB analysis.

Analysis for Total Substituted Urea Residues. Total residues, hydrolyzable to DCA, were determined by the method described by Pease (1962).

Gas Chromatographic Analysis for Tetrachloroazobenzene. An aliquot of the hexane extract of soil was injected after equilibration of the gas chromatograph as described under Calibration. The micrograms of TCAB were determined from a calibration curve prepared as described under Calibration. The TCAB content in terms of part per million was calculated by dividing the micrograms found, corrected for aliquot and recovery factors, by sample weight in grams. Typical gas chromatograms of extracts of a diuron, DCA (1:1 dilution), and propanil (1:50 dilution) treated soil along with that of an untreated soil are illustrated in Figure 2.

Because of the fact that linuron partitions into the hexane phase during extraction, it was necessary to adjust the program rate to 2° C. per minute over a temperature range of 100° to 190° C., to separate interferences due to linuron and its possible degradation products for the analysis of extracts of linuron-treated soils. Under these conditions, the retention time for TCAB was approximately 32 minutes. It was also necessary with these particular extracts to leave the vent open for 30 minutes after start of temperature programming to bypass the interferences away from the titration cell. Chromatograms illustrating the recovery of TCAB under these special conditions are shown in Figure 3.

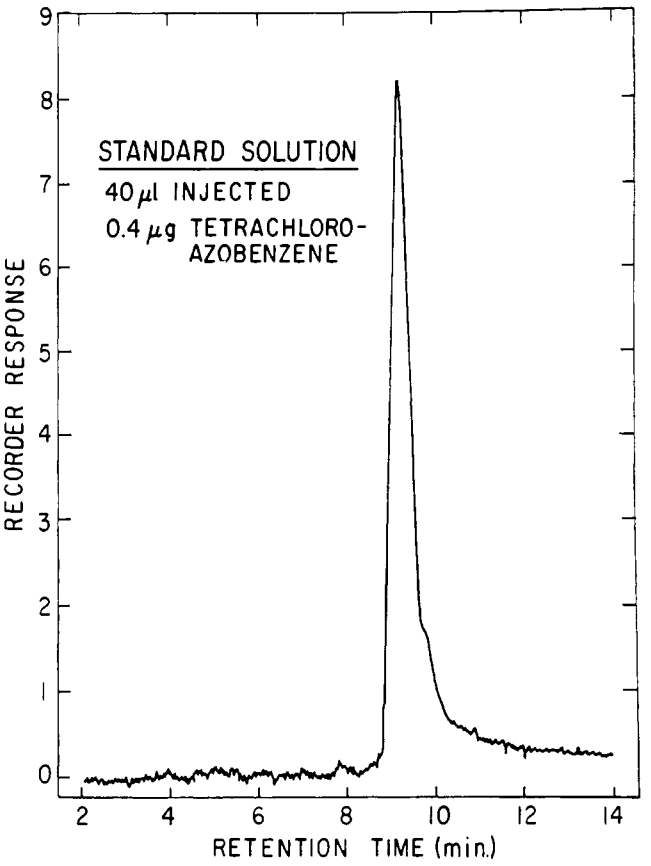

Figure 1. Gas chromatogram of a 3,3′,4,4′-tetrachloroazobenzene standard solution

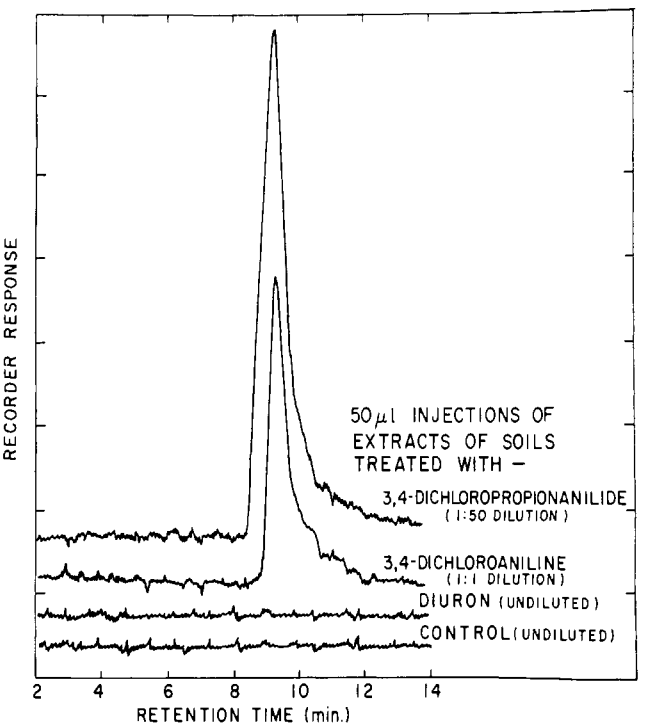

Figure 2. Gas chromatograms of extracts of soils incubated with 3,4-dichloropropionanilide, 3,4-dichloroaniline, and diuron

Colorimetric Analysis for Dichloroaniline. The DCA content of soil extracts was determined by the method described

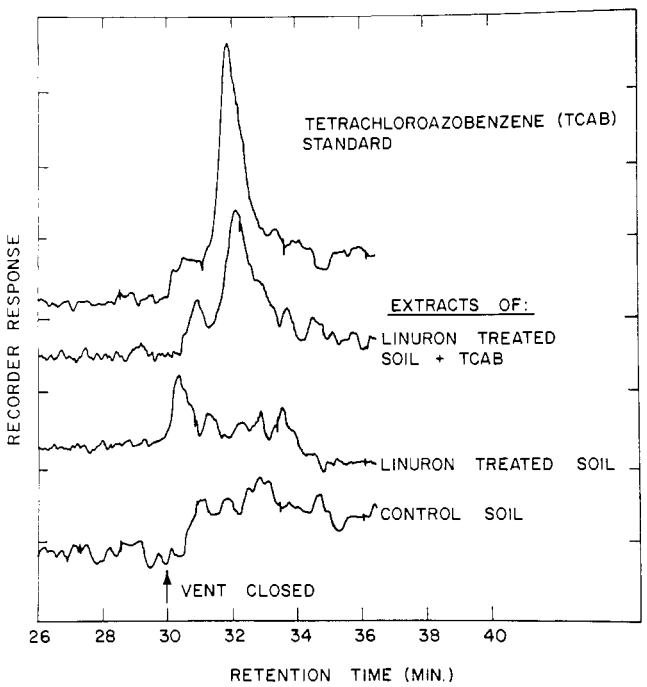

Figure 3. Gas chromatograms of a 3,3′,4,4′-tetrachloroazobenzene standard solution, and an extract of a soil incubated with linuron

Table I. Recovery of Tetrachloroazobenzene Added to Soil
(50-Gram Samples)

TCAB Concn., p.p.m.	TCAB, μg. Added	TCAB, μg. Found	Recovery, %	Remarks
0.1	5	4.1	83	TCAB in contact
0.3	15	13.1	87	with soil 1 hr.
0.5	25	23.5	94	before extraction
0.1	5	3.8	76	TCAB in contact
0.3	15	12.2	82	with soil 24 hrs.
0.5	25	21.2	85	before extraction

by Pease (1962) which involves the diazotization and coupling reaction of DCA.

RESULTS AND DISCUSSION

Initially, the recovery of TCAB 1 and 24 hours after addition to moist soil was determined. The data shown in Table I indicate that the extraction and the gas chromatographic procedure used for TCAB in soil gave excellent recoveries down to at least 0.1 p.p.m. Also, it appears evident that the recovery of TCAB after 24 hours of exposure to soil was slightly less than that experienced after 1 hour of exposure.

Analysis of a Keyport-type field soil which had been treated with diuron for 12 consecutive years at annual rates of 2 and 4 pounds per acre showed no detectable levels of TCAB or DCA (Table II). The total residues hydrolyzable to dichloroaniline were found to be 3.9 and 5.8 p.p.m. (calculated as apparent diuron) for the 2 and 4 pound per acre treatments, respectively. These diuron residue values are of the same order of magnitude as those reported by Dalton *et al.* (1966). However, they reported minimal levels (0.3–0.8 p.p.m.) of DCA in their soil samples, using a more exhaustive extraction procedure. Similarly, a soil sample taken from a field plot approximately 2 months after a 2-pound per acre treatment with linuron showed no detectable DCA or TCAB, using the

Table II. Analysis of Field Soils for Herbicide Residues
(50-Gram Samples)

Diuron Treated Plots

12-Yr. Annual Diuron Rate,[a] lbs./A	Apparent[c] Diuron, p.p.m.	DCA, p.p.m.	TCAB, p.p.m.
0	<0.1	<0.1	<0.1
2	3.9	<0.1	<0.1
4	5.8	<0.1	<0.1

Linuron Treated Plot

Linuron[b] Rate, lbs/A	Apparent[c] Linuron, p.p.m.	DCA, p.p.m.	TCAB, p.p.m.
0	<0.1	<0.1	<0.1
2	1.6	<0.1	<0.1

[a] Last diuron application, June 1966. Sample taken 6/27/67.
[b] Date of linuron application, 6/6/67. Sample taken 8/3/67.
[c] Total residual material hydrolyzable to 3,4-dichloroaniline calculated as apparent diuron or linuron.

Table III. Analysis of Laboratory Soils for Herbicide Residues
(50-Gram Samples)

Treatment	Initial Concentration p.p.m.	Residual Concentration, p.p.m. DCA After 14 Days	DCA After 31 Days	TCAB After 14 Days	TCAB After 31 Days
Check	—	<0.1	<0.1	<0.1	<0.1
3,4-Dichloropropionanilide	500	135	121	105	143
Diuron	500	0.9	0.5	<0.1	<0.1
Linuron	500	1.4	0.5	<0.1	<0.1

extraction conditions described by Bartha and Pramer (1967). The apparent linuron content of the soil was 1.6 p.p.m. (Table II).

In addition to the analyses of the above field soils, a test was patterned after that described by Bartha and Pramer (1967) to determine whether diuron or linuron, when incubated in soil under such test conditions, would yield TCAB. Included in this experiment were laboratory soils with propanil. The results of this test, summarized in Table III, confirmed the findings of Bartha and Pramer in that propanil was found to degrade to give appreciable quantities of DCA and TCAB. The values obtained for both DCA and TCAB were in good agreement with those reported by the above investigators. In contrast, however, both the diuron- and linuron-treated soils under the same experimental conditions showed only approximately 1 p.p.m. or less of DCA after 14 and 31 days of incubation and no detectable TCAB (<0.1 p.p.m.).

In two separate follow-up experiments, diuron was re-evaluated as a precursor to TCAB in soil. In both experiments, dichloroaniline was also tested at various concentrations in the soil as a TCAB precursor. The results shown in Table IV again demonstrated that diuron at a high soil concentration of 500 p.p.m. (equivalent to a field treatment of 500 pounds per acre to a depth of 3 inches) failed to yield detectable levels of TCAB in the soil. Surprising, however, was the finding that DCA treatments of the soil failed to yield the anticipated soil concentrations of TCAB, after 31 days of incubation. This was more surprising since the residual soil concentrations of DCA after 31 days in the flasks initially treated with 200 to 500 mg. of DCA were of the same order

Table IV. Analysis for Dichloroaniline and Tetrachloroazobenzene in Laboratory Soils Treated with Diuron and Dichloroaniline

(50-Gram Samples)

Treatment	Initial p.p.m.	Concentration after 31 days of DCA, p.p.m.	Concentration after 31 days of TCAB, p.p.m.
Check	—	<0.1	<0.1
Diuron	500	0.7	<0.1
3,4-Dichloroaniline	500	210.0	2.7
	200	71.0	2.3
	100	15.0	5.4
	50	4.0	1.3
	20	0.9	0.2
	10	0.5	0.1
Check	—	<0.1	<0.1
Diuron	500	0.5	<0.1
3,4-Dichloroaniline	500	235.0	2.4
	300	127.5	2.6
	200	68.5	4.5

Table V. Recovery of Dichloroaniline (DCA) 24 Hours after Addition to Soil

(50-Gram Samples)

DCA Added, p.p.m.	DCA Found, p.p.m.	Recovery, %
500	384.0	77
200	146.0	73
100	69.6	70
50	31.6	63
20	12.2	61

Table VI. Analysis for Dichloroaniline and Tetrachloroazobenzene in Laboratory Soils Treated with 2,4-Dichloropropionanilide and 3,4-Dichloroaniline

(50-Gram Samples)

Treatment	Initial Concentration, p.p.m.	Concentration after 42 Days of DCA, p.p.m.	Concentration after 42 Days of TCAB, p.p.m.
Check	—	<0.1	<0.1
3,4-Dichloropropionanilide	500	94.6	83.5
	250	36.0	35.4
3,4-Dichloroaniline	500	136.6	4.5
	300	66.6	2.4
	200	36.6	1.8
	100	13.2	1.0

of magnitude as found in the propanil-treated soils of the previous experiment (Table III). The DCA-treated soils contained no more than 5% of the TCAB found in the comparable propanil-treated soils.

A recovery study was made to determine volatile DCA losses from soil during the first 24-hour period prior to the adjustment of the soil moisture content (8.2%) to 50% of its total water holding capacity. The losses (Table V) ranged from 23% for the 500 p.p.m. DCA treatment to 39% for the 20 p.p.m. treatment. Comparison of the residual DCA concentrations in the soil after 24 hours with those after 31 and 42 days (Tables IV and VI) indicate that further losses had occurred but at a much slower rate. These apparent interim losses were probably due to the binding capacity of the soil and microbial action in addition to volatilization.

In a final experiment, 3,4-dichloropropionanilide at soil concentrations of 250 and 500 p.p.m. was compared directly with 3,4-dichloroaniline at initial soil concentrations of 100 to 500 p.p.m. The soils were incubated over a 42-day period before extraction and analysis. The results of this test (Table VI) confirm the earlier findings. At the initial propanil levels of 250 and 500 p.p.m., the final DCA concentrations were 36.0 and 94.6 p.p.m., respectively, and the final TCAB concentrations were 35.4 and 83.5 p.p.m., respectively. In contrast, however, DCA soil treatment, under the same laboratory conditions, failed to yield comparable concentrations of TCAB. The TCAB concentrations found in soils treated with DCA were but a fraction of those found in the propanil-treated soils, even though the final DCA soil concentrations were comparable for both treatments.

These data, therefore, indicate that the dichloroaniline is probably not the prime precursor of tetrachloroazobenzene. If it were, then the concentration of TCAB found in soil would have been proportional to the dichloroaniline content of soil.

ACKNOWLEDGMENT

The authors are grateful to R. W. Luckenbaugh for his valuable suggestions throughout the program, and to F. J. Baude, who synthesized the tetrachloroazobenzene used in this study.

LITERATURE CITED

Bartha, R., J. Agr. Food Chem. **16,** 602 (1968).
Bartha, R., Linke, H. A. B., Pramer, D., *Science* **161,** 582 (1968).
Bartha, R., Pramer, D., *Science* **156,** 1617 (1967).
Dalton, R. L., Evans, A. W., Rhodes, R. C., *Weeds* **14,** 31 (1966).
Gaudry, R., Keirsted, K. F., *Can. J. Res.* **27B,** 897 (1949).
Geissbühler, H., Haselbach, C., Aebi, H., Ebner, L., *Weed Res.* **3,** 277 (1963).
Hill, G. D., Evans, A. W., Varner, R. W., *Proc. Northeastern Weed Control Conf.* **16,** 348 (1962).
Hill, G. D., McGahen, J. W., Baker, H. M., Finnerty, D. W., Bingeman, C. W., *Agron. J.* **47,** 93 (1955).
Kearney, P. C., Kaufman, D. D., "Soil Biochemistry," Marcel Dekker, New York, 1967.
Kearney, P. C., Plimmer, J. P., Guardia, F. B., *Abstracts, Weed Soc. Amer.*, Feb. 1969.
Lode, O., *Weed Res.* **7,** 185 (1967).
Nash, R. G., *Agron. J.* **60,** 177 (1968).
Pease, H. L., *J. Assoc. Offic. Agr. Chem.* **45,** 377 (1962).
Smith, J. W., Sheets, T. J., J. Agr. Food Chem. **15,** 577 (1967).

I. J. Belasco
H. L. Pease

Research Division
Industrial and Biochemicals Department
E. I. du Pont de Nemours and Company
Wilmington, Del. 19898

Received for review March 12, 1969. Accepted June 10, 1969.