Chromatographic Procedure for the Determination of Maloran and Metabolites in Soils

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Maloran, 3-(3-chloro, 4-bromophenyl)-1-methoxy-1-methylurea, is a selective herbicide nematicide used for weed control in corn. The substituted urea herbicides as a group are degraded in the soil by microbial action (3,4,7) with minimal accumulation of phytotoxic residues. Geissbuhler et al. (3) showed that cluroxuron, 3-(p-chlorophenoxyphenyl)-1, 1-dimethylurea was degraded in soil by successive demetylation to the urea followed by hydrolysis of the urea to the aniline. This mechanism of degradation was confirmed by Dalton et al. (2) in their study of the disappearance of diuron,3-(3,4 dichlorophenyl)-1, 1-dimethylurea from cotton field soils.

Bulletin of Environmental Contamination & Toxicology,
Vol. 3, No. 5, 1968, published by Springer-Verlag New York Inc.

The procedure presented in this report employs both thin-layer and gas chromatographic techniques that can be used in laboratory studies in conjunction with the Bartha and Pramer (1) procedure or with the Kaufman perfusion apparatus (6) for determining biodegradation and the mechanism of breakdown in the soil. Both procedures presented can be used qualitatively and quantitatively for the microbial degradation of this herbicide in laboratory studies.

Materials and Methods

Reagents

a-Ethyl acetate and hexane-Nanograde or Pesticide Quality

b-Chloroform, acetone, and pyridine-ACS grade or equivalent redistilled before use

c-Mitchell's reagent - dissolve 1.7 g. silver nitrate in 5 ml. of water. Add 20 ml. of 2-phenoxyethanol (Eastman P 4861) and dilute with acetone to 200 ml. Add 1 drop of 30% hydrogen peroxide and mix. Allow reagent to stand overnight before use. Store in a dark bottle.

d-Eastman chromatogram sheets K301R2 100u silica gel without flourescent indicator.

e-Solvent System A-Hexane: Ethyl Acetate 15:2v/v

f-Solvent System B- Chloroform:Pyridine 10:1v/v

g-Standard Solutions - Prepared to contain 1 mg./ml. ethyl acetate. Any necessary dilutions are made from these solutions.

Equipment

a- Gas chromatograph - MicroTek Mt-220 equipped with a flame ionization detector and linear temperature programmer.

b- Chromatographic column - 16 inch x 1/4 inch O.D. glass column packed with 1.5 % XE-60 on Gas Chrom Q 80-100 mesh. This column was aged at 240 C for 48 hr. with a nitrogen flow of 70 ml./min.

c- DrummondMicrocaps 5 ul. Drummond Scientific Company, Broomall, Pennsylvania.

d- Ultraviolet light such as Uvi-Arc Model 420-U 1 Raymaster.

Compounds

a- 3-(3-chloro,4-bromophenyl)-1-methoxyl-1-methylurea

b- 3-(3-chloro,4-bromophenyl)-1-methoxyurea

c- 3-(3-chloro,4-bromophenyl)-1-methylurea

d- 3-(3-chloro,4-bromophenyl) urea

e- 3 chloro,4 bromoaniline

Soil Types

Designation	Type
a- Parsippany	Silty Clay Loam
b- Lakewood	Sand
c- Norton	Silt Loam
d- Colts Neck	Sandy Loam
e- Dutchess	Shale Loam
f- Bermudian	Silt Loam
g- Annandale	Loam

Procedure

Soil samples were screened to pass a 20 mesh screen. One hundred gram samples of 7 soils were used for supplementation with similar 100 gram samples of each soil used as controls. Ten mg. of maloran and each of its metabolites were dissolved in ethanol and added to each of the soil samples. To each of the soil samples, 20 ml. of water was added to bring the water content to approximately 18%. Each soil sample was thoroughly mixed and allowed to stand in an open evaporating dish for 3 days to air-dry. The soil samples were removed from the evaporating dish and placed into an extraction thimble and extracted using a sohxlet extractor for 6 hr. using 250 ml. ethyl acetate as the extracting solvent.

The ethyl acetate was acidified with 2 ml. of glacial acetic acid and reduced in volume on a steam bath with the aid of a stream of air or with a Danish Kuderna concentrator to a volume of 5 ml.

Fifteen ul. of soil extract containing the maloran and metabolites was spotted on the Eastman thin-layer sheet. Similarly, the extracts of unsupplemented soils were spotted. Five ul. of a mixture of standards were also spotted and the plate was developed twice in solvent system A. The plate was removed from the developing tank and traces of the solvent mixture were removed by means of a hair dryer. The plate was chromatographed once more in solvent system B. After development, the plate was dried again with the aid of the hair dryer. The plate was sprayed with Mitchell's reagent and visualized under a ultraviolet lamp.

To obtain confirming G L C data, thin-layer chromatograms were sprayed only on that portion of the plate spotted with standards. Those areas corresponding to standards were removed, extracted with 5 ml of ethyl acetate and the volume reduced to 1 ml. Five ul. of ethyl acetate extract was injected into the chromatograph. The G L C parameters used were: injector temperature - 190 C, initial coumn temperature 75 C, program rate - 5 C/min.

final column temperature 230 C, nitrogen flow 70 ml./ min., hydrogen flow 30 ml./min., and air flow 1 CFM. Retention times and relative retention times of the areas removed from the thin-layer chromatograms were compared with standards similarly chromatographed.

Results and Discussion

Maloran and its suspected soil metabolites were equilibrated with 7 soils for 3 days prior to extraction. This time period was far in excess of the 0.5 hr. found by Sherbourne and Freed (8) necessary to accomplish equilibrium for monuron with the colloidal and organic fractions of soil. The 3 day time interval is quite reasonable since 3 days are required to bring samples to the point where extraction can be performed.

The 6 hr. extraction with ethyl acetate appears to be sufficient to remove the supplemented compounds from the soils. The addition of glacial acetic acid to the extract prevents the loss of the 3-chloro,4-bromoaniline which had the tendency to co-distill during the volume reducing step. Table 1 shows the Rf values found with standards co-chromatographed with soil extracts on a typical thin-layer chromatogram.

TABLE 1

Chromatographic Data for Maloran and Metabolites

	Rf Values		
Compound	Mix 1	Mix 2	Mix 3
Maloran	0.93	0.93	0.93
3-chloro,4-bromoaniline	0.80	0.80	0.79
3-(3-chloro,4-bromophenyl) -1-methoxyurea	0.71	0.71	0.71
3-(3-chloro,4-bromophenyl) -1-methylurea	0.42	0.42	0.43
3-(3-chloro,4-bromophenyl) urea	0.17	0.17	0.18

Table 2 shows the Rf values obtained from the soils supplemented with maloran and metabolites. The values obtained from soil extracts agree quite well with those of the standards.

TABLE 2

Chromatographic Data for Maloran and Metabolites in Several Soils

Compounds	Soils						
	1	2	3	4	5	6	7
Maloran	0.88	0.91	0.93	0.93	0.93	0.93	0.93
3-chloro,4-bromo aniline	0.74	0.78	0.79	0.79	0.80	0.81	0.78
3-(3-chloro,4-bromo phenyl)-1-methoxyurea	0.65	0.68	0.68	0.70	0.72	0.72	0.71
3-(3-chloro,4-bromo phenyl)-1-methylurea	0.39	0.39	0.40	0.41	0.43	0.43	0.42
3-(3-chloro,4-bromo phenyl) urea	0.15	0.16	0.16	0.15	0.18	0.16	0.15

1-Annandale
2-Bermudian
3-Dutchess
4-Colts Neck
5-Norton
6-Lakewood
7-Parsippiny

Table 3 shows the retention time and relative retention time of maloran and metabolites with the exception of 3-(3-chloro,4-bromophenyl)urea which cannot be determined by gas chromatography.

TABLE 3

Gas Chromatographic Data for Maloran and Metabolites

Compound	Retention Time in Min.	Relative Retention Time
Maloran	5.20	1.00
3-chloro,4-bromoaniline	2.70	0.52
3-(3-chloro,4-bromophenyl) -1-methoxyurea	6.18	1.19
3-(3-chloro,4-bromophenyl) -1-methylurea	7.48	1.44

The areas removed from thin-layer plates were extracted and analyzed by gas chromotography. Table 4 shows the results obtained with standards and compounds obtained from the supplemented soils.

TABLE 4

Gas Chromatographic Comparisons of Standards With Compounds Separated from Supplemented Soils

Compound	Source	Ret.Time Min.	Ret.Time Maloran	Rel.Ret Time
3-chloro,4-bromoaniline	Std.	2.70	5.10	0.53
	Soil	2.75	5.10	0.53
3-(3-chloro,4-bromophenyl) -1-methoxyurea	Std.	6.15	5.20	1.16
	Soil	6.05	5.15	1.17
3-(3-chloro,4-bromophenyl) -1-methylurea	Std.	7.40	5.20	1.42
	Soil	7.20	5.05	1.43

Confirmation of the identity of the compounds can be made by use of micro infrared techniques. The thin layer procedure lends itself to the use of radioisotopes.

Adaptation of the Kaufman perfusion technique requires a 16 hr. liquid-liquid extraction using ethyl acetate as the solvent. Otherwise, the techniques are the same.

The intent of this procedure is to provide the methodology to follow the mechanism of biodegradation in soils under laboratory conditions. Adaptation of this procedure to field samples or to soil samples of unknown origin was not undertaken.

Acknowledgement

The authors would like to acknowledge the supplying of analytical standards by V. Frank Boyd, CIBA Agro-Chemical Corporation, Vero Beach, Florida.

References

1. Bartha,R., and Pramer,D., Soil Sci. 100,68 (1965)

2. Dalton,R.L., Evans,A.W., and Rhodes,R.C., Weeds 14, 31 (1966)

3. Geissbuhler,H., Haselbach,C., Aebi,H., and Ebner,L., Weed Res. 3,277 (1963)

4. Hill,G.D., MaGahen,J.W., Baker,H.M., Finnerty,D.W., and Bingeman,C.W., Agron J. 47,93 (1955)

5. Kaufman,D.D., Weeds 14,90 (1966)

6. Mitchell,L.C., J. Assoc. Offic. Agr. Chemists 44, 644 (1961)

7. Sheets,J.J., J. Agr. Food Chem. 12,30 (1964)

8. Sherbourne,R.R., and Freed,V.H., J. Agr. Food Chem. 2,939 (1954)

Paper of the Journal Series, New Jersey Agriculture Experiment Station. These investigations were supported in part by U.S. Public Health Research Grant ES-00016-02.