Fungicide Movement in Soils

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ABSTRACT

Bioassay methods, in combination with soil thin-layer chromatography (soil TLC), were developed to assess mobility of fungicides in soils. After leaching soil TLC plates with water, 10 soil fungi (*Aspergillus fumigatus, Diplodia zeae,* two isolates of *Fusarium moniliforme, Fusarium roseum, Helminthosporium sativum, Penicillium chrysogenum, Penicillium rugulosum, Rhizoctonia solani,* and *Trichoderma viride*), and an alga (*Chlorella sorokiniana*) were tested as visualizing agents by spraying plates with a liquefied nutrient agar suspension of the organism. Plates were incubated at 100% RH and approx. 28 C until zones of inhibition or stimulation appeared, usually in 1 to 4 days. The movement of 38 pesticides (33 fungicides, 3 insecticides, 1 acaricide, and 1 herbicide) in Hagerstown silty clay loam was determined. Relatively mobile compounds included: cycloheximide, cycloheximide oxime, Ceresan L (the mercaptide component), Dexon, formetanate, formparanate, and oxycarboxin. Immobile compounds included chloranil, chloroneb, DCNA, dichlone, dodine, hexachlorophene, Morestan, PCNB, TCNA, Terrazole, and zineb. *T. viride* and *C. sorokiniana* were the two organisms sensitive to the greatest number of fungicides. The mobility order nabam > maneb > zineb was confirmed by bioassay and autoradiography. In five soils, movement of these [^{14}C]dithiocarbamate fungicides was inversely related to soil organic matter content.

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Additional key words: fungicide diffusion, organic mercurial fungicides, dithiocarbamate fungicides.

Fungicide movement in soils may be a mechanism of loss or a means of tranporting active chemical to the target pathogen. The degree of mobility, which now often governs the type of application, may eventually influence the formulation selected. For example, acid surfactants were recently added to benomyl (34) to increase its soil mobility, since control of Verticillium wilt of cotton was proportional to the depth of physical incorporation of the fungicide (26).

In a review of physical aspects of soil which affect fungicide behavior, Goring's (10) references to fungicide movement related almost entirely to the vapor phase, i.e., to fumigants. By comparison with herbicides, few studies of the physical behavior of nonvolatile fungicides are published. Early interest was focused on soil drenches; efficacy leads, a priori, to an assumption of some fungicide transport. The use of soil columns was an improvement over agar plate efficacy tests, which failed to measure soil mobility, which is necessary information to determine whether the chemical must be applied as a drench, injected below the surface, or mixed mechanically into soil (25). Munnecke (28) evaluated movement of 13 nonvolatile fungicides in soil columns and concluded that penetration was greater for solution formulations vs. suspensions; for finer fungicide particles vs. coarse particles; for coarser-textured soils; and for initially dry soils.

The leaching of pesticides in soils has been considered as a chromatographic process by a number of workers (31). It is recognized that physical transport in nature is extremely complex because of soil physical and chemical nonhomogeneity, continually variable moisture parameters, and biological interactions. Nonetheless, the simplified approach has merit for evaluating basic factors affecting pesticide adsorption and movement.

The soil thin-layer chromatographic (soil TLC) technique, developed in 1968 by Helling and Turner (21), has proved useful in studies of relative mobility of pesticides (15) and the effects of soil properties thereon (14, 16). Water moves in the soil TLC layer by unsaturated flow (14), resembling typical water flow in the field. The present paper describes a fungal bioassay developed for use with soil TLC, and the subsequent evaluation of 38 pesticides (including 33 fungicides) in terms of leaching and diffusion characteristics.

MATERIALS AND METHODS.—Hagerstown silty clay loam, a surface soil from Maryland, contained 2.50% organic matter and 39.5% clay; the pH was 6.8. The soil water content at a tension of 0.33 bar was 34.1%.

The fungicides tested, their sources, and forms are listed in Table 1.

Test organisms.—One isolate of each of the following fungi was used: *Aspergillus fumigatus* Fres., *Diplodia zeae* (Schw.) Lev., *Fusarium roseum* f. *cerealis* (Cke.) Snyd. & Hans., *Helminthosporium sativum* Pam., King & Bakke, *Penicillium chrysogenum* Thom, *Penicillium rugulosum* Thom, *Rhizoctonia solani* Kuhn, and *Trichoderma viride* Pers. ex Fr. Two isolates of *Fusarium moniliforme* (Sheldon) Snyd. & Hans., designated as #125 and #412, were also used. Cultures were usually grown on Czapek's medium, which had the following composition: 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4 \cdot 7H_2O$, 30 g sucrose, and 1,000 ml distilled water.

The alga *Chlorella sorokiniana* Shihira & Krauss (strain 7-11-05) was maintained and used as previously described (19).

Soil TLC.—The basic technique has been described (14, 21). Dry soil was sieved to <250 μm (coarse-textured soils were sieved to <500 μm), moistened with water until moderately fluid, then applied to glass TLC plates with a commercial TLC spreader. Usually the Hagerstown soil layer thickness was 500 μm for detection by autoradiography, or 1,000 μm for bioassays.

Pesticides were applied at 1.5 cm above the base and the

plate leached 10 cm with water in a closed chamber. Plates were air-dried after the 2- to 3-h development. Duplicate pesticide applications were often made in the upper nonleached zone to assess diffusion which occurs during the bioassay.

When radioactive pesticides were used, no-screen medical X-ray film was placed in direct contact with the soil plate for ⩾3 days. Movement was then recorded as the frontal R_f of a spot or streak.

Bioassay.—Nonradioactive pesticides were detected by bioassay. Actively growing fungal cultures, in liquid medium, were collected by filtering the mycelial mass in a Buchner funnel, fragmenting the tissue in fresh medium in a blender, filtering through cheesecloth, and collecting the filtrate which contained spores and mycelial fragments. Typically, 50 ml of fungal suspension was processed, resuspended in 50 ml fresh medium, and warmed to 45 C. This was combined with 50 ml of

TABLE 1. Fungicides tested and their sources

Fungicide[a]	Chemical name	Source	Purity[b]
ACNQ	2-amino-3-chloro-1,4-naphthoquinone	U.S. Rubber	Anal.
Benomyl	methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate	DuPont	Tech.
Binapacryl	2-*sec*-butyl-4,6-dinitrophenyl 3-methyl-2-butenoate	Niagara	98.7%
Captan	*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide	Stauffer	94%
Ceresan L®	methylmercuric 2,3-dihydroxypropylmercaptide (2.89%) + methylmercuric acetate (0.62%)	DuPont	3.51%
Ceresan M®	*N*-(ethylmercuri)-*p*-toluenesulfonanilide	DuPont	7.7%
Chloranil	tetrachloro-*p*-benzoquinone	U.S. Rubber	Anal.
Chloroneb	1,4-dichloro-2,5-dimethoxybenzene	DuPont	Tech.
Cycloheximide (Acti-dione®)	3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide	Upjohn	85-100%
Cycloheximide oxime	3- 2-[3,5-dimethyl-2-(hydroxyimino)cyclohexyl]-2-hydroxyethyl glutarimide	Upjohn	Anal.
D198	2,5-dimethyl-1,4-benzoquinone monoxime	U.S. Rubber	Anal.
DCNA	2,6-dichloro-4-nitroaniline	Upjohn	100%
Dexon® (Diazoben)	*p*-dimethylaminobenzenediazo sodium sulfonate	Chemagro	96.2%
Dichlone	2,3-dichloro-1,4-naphthoquinone	U.S. Rubber	Anal.
Disulfoton	*O,O*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate	Chemagro	98%
Dodine	*n*-dodecylguanidine acetate	Amer. Cyanamid	100%
Dyrene®	2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine	Chemagro	96%
E-275	stendomycin salicylate	Eli Lilly	2.52% (EC)
Ferbam	ferric dimethyldithiocarbamate	DuPont	76% & Anal.
Formetanate hydrochloride	*m*- [(dimethylamino)methylene]amino phenyl methylcarbamate hydrochloride	Schering	94%
Formparanate hydrochloride	4- [(dimethylamino)methylene]amino -*m*-tolyl methylcarbamate hydrochloride	Schering	92%
Hexachlorophene	2,2′-methylenebis(3,4,6-trichlorophenol)	Nationwide Chem.	Tech.
Karathane® (Dinocap)	2,4-dinitro-6-octylphenyl crotonate	Rohm & Haas	Purified
Maneb	manganous ethylenebis(dithiocarbamate)	DuPont	80%
Morestan®	6-methyl-1,3-dithiolo[4,5-b]quinoxalin-2-one	Chemagro	92%
Nabam	disodium ethylenebis(dithiocarbamate)	DuPont	22% & purified[c]
Oxycarboxin (Plantvax®)	5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide, 4,4-dioxide	U.S. Rubber	Anal.
Panogen 15®	cyano(methylmercuri)guanidine	Morton	2.2%
PCNB	pentachloronitrobenzene	Olin Mathieson	Tech.
PCP	pentachlorophenol	Dow	86%
PCP-(Na)	sodium pentachlorophenate	Dow	79%
Phenmedipham	methyl *m*-hydroxycarbanilate *m*-methylcarbanilate	Schering	>95%
Promecarb	2-methyl-5-isopropylphenyl methylcarbamate	Schering	>98%
TCNA	2,3,5,6-tetrachloro-4-nitroanisole	Smith, Kline & French	Tech.
Terrazole®	5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole	Olin	95%
Thiram	bis(dimethylthiocarbamoyl) disulfide	DuPont	Anal.
Zineb	zinc ethylenebis(dithiocarbamate)	DuPont	75% & Anal.
Ziram	zinc dimethyldithiocarbamate	DuPont	76% & Anal.

[a]Nonfungicides are listed as a footnote to Table 2.
[b]Manufacturer's designation of purity.
[c]Synthesized by C. L. Fletcher, U.S.D.A., and recrystallized from aqueous acetone.

medium which contained double-strength agar (30 g/liter), previously cooled to 45 C. The mixture was sprayed immediately onto a 20-×20-cm leached soil TLC plate. Plates were incubated at approx. 28 C and 100% relative humidity (RH), in darkness. Zones of inhibition or stimulation usually appeared within 16 h in actively growing cultures, but plates were often observed for 3 to 4 days to confirm results.

The fungal bioassay was easily adapted to large-scale operations. For example, testing of all compounds, each with four replications, required 40 plates. For this, 10 liters of liquid medium inoculated with an organism was cultured in a batch fermenter with aeration. Harvesting and spraying were accomplished during one day.

Techniques for the algal bioassay were similar, the principal differences being that cells were harvested by centrifugation and incubation was conducted in the light. Helling et al. (19) described the use of this bioassay in detail.

RESULTS AND DISCUSSION.—*Use of fungal bioassays in chromatography.*—The fungal bioautography technique was first reported in 1958. Weltzien (40) chromatographed thiram, ferbam, captan, and other fungicides on paper, then sprayed the dried chromatogram with a suspension of *Stemphylium consortiale* in nutrient solution, and incubated 72 h. Fungitoxic spots became visible as clear areas on a background opaque with fungal growth. Others subsequently used this technique, as in studies of sodium dimethyldithiocarbamate (6, 7), or instead incubated the unsprayed paper in contact with a seeded nutrient agar plate (3).

Thin-layer chromatography has largely superseded paper chromatography, and the fungal bioassay has been useful for visualizing separations on TLC plates (9, 22, 33). One modification has been to coat the leached plate with warm biomalt agar, inoculated with a spore suspension, before incubating (4). Organisms vary markedly in their susceptibility to chemicals, but Fuchs et al. (9) found that use of *Cladosporium cucumerinum* permitted detection of as little as 0.01 μg of fungitoxicant.

Use of fungal bioassays in soil TLC.—Soon after our first report on a fungal bioassay for soil TLC studies (18), Stipes and Oderwald (38) briefly described a similar method for visualizing benomyl, captan, and thiabendazole movement in soil. They sprayed *Penicillium expansum* conidia on developed soil TLC plates. Additional work (17) has resulted in the information described herein.

Static diffusion experiments.—Ten isolates of fungi, listed previously, were chosen for testing because all were common soil pathogens. We did not search for unusually sensitive strains of fungi because (i) we first wished to assess the feasibility of a fungi bioassay for soil TLC, (ii) we needed inherent mobility data, and (iii) we were testing a very large and diverse group of chemicals. Highly sensitive or specific strains, as have been used on conventional chromatograms, are certainly recommended when applying the soil TLC technique to only a few homologous fungicides.

Fungicides were first screened by a method designed to show both toxicity of a chemical and its diffusion in soil. One hundred micrograms of each fungicide was applied within 5-cm squares (more volatile compounds were added last), scribed into the soil layer, and the soil plates were bioassayed as described earlier. Every chemical was tested with one or more of the following organisms: *A. fumigatus, F. roseum, P. chrysogenum, P. rugulosum,* and *T. viride.*

These preliminary experiments indicated extensive radial diffusion (at least 2.0 - 2.5 cm) of Panogen 15, Ceresan L, Ceresan M, and cycloheximide during the bioassay. Among the compounds diffusing least were dodine, hexachlorophene, chloranil, E-275, cycloheximide oxime, and Terrazole. Diffusion was not obviously related to leachability (determined later); the significant movement recorded for fungicides such as maneb or ferbam is likely, as leaching experiments suggested, to reflect diffusible toxic contaminants rather than the parent compound. These experiments also demonstrated that compounds sometimes stimulate, rather than inhibit, mycelial growth; this often occurred only on the perimeter. Two explanations seem plausible: either low concns of some chemicals enhance growth of certain fungi (perhaps by concn-dependent activation or inhibition of enzymes), or diffusive, stimulatory contaminants are present. Stimulation as well as inhibition was useful for visualization in leaching experiments.

Leaching experiments (bioassay).—We initially tested various methods of adding fungi after leaching, including: directly spraying a fungal suspension in nutrient medium; pouring agar onto the soil plate either before or after spraying the fungi; and transferring a cooled agar slab (containing fungal spores) onto the leached soil plate.

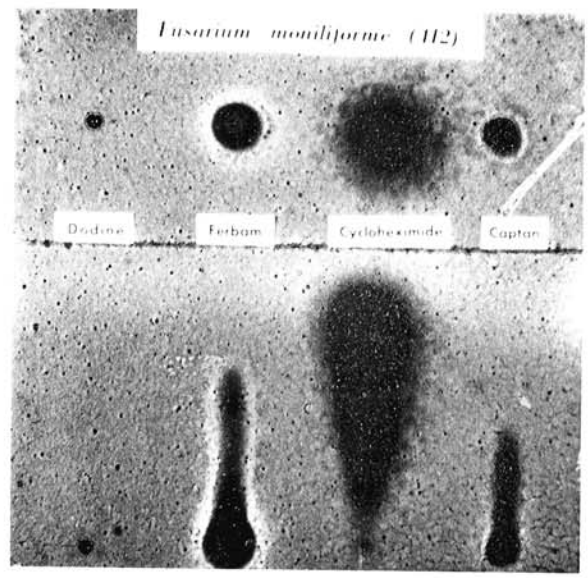

Fig. 1. Movement of dodine, ferbam, cycloheximide, and captan fungicides in a Hagerstown silty clay loam TLC plate. Visualization is by fungal bioassay using *Fusarium moniliforme* (isolate #412). The upper portion of the plate was nonleached and shows movement by diffusion only; the lower 11.5 cm was leached.

TABLE 2. Fungicide mobility in Hagerstown silty clay loam soil TLC plates

Fungicide[a]	Average[b] R_f (× 100) using indicated bioassay organism[c]											Overall mean[d] R_f
	Af	*Dz*	*Fm1*	*Fm4*	*Fr*	*Hs*	*Pc*	*Pr*	*Rs*	*Tv*	*Cs*	
ACNQ	5	5	11	9	13	7	7	2	10	10	12	0.08
Benomyl	22	23	19	18	...	...	22	16	...	19	15	0.19
Binapacryl	[48]	...	...	47	...	...	48	...	[43]	61	48	0.49
	1	3	6	8	1	0	6	3	[2]	2	1	0.03
Captan	40	41	46	38	40	34	42	36	38	39	37	0.39
	ND	ND	1	ND	5	1	ND	3	2	1	ND	0.02
Ceresan L	91	88	91	83	85	85	87	87	86	95	90	0.88[e]
	ND	ND	7	ND	ND	ND	ND	ND	ND	ND	ND	0.07
Ceresan M	5	8	3	2	5	3	4	3	5	4	13	0.05
Chloranil	1	0	0	1	...	0	[1]	0	0	1	1	0.00
Chloroneb	...	...	1	...	0	2	0	0	...	1	[0]	0.01
Cycloheximide	89	91	90	89	89	90	85	...	88	92	91	0.89
Cycloheximide oxime	83	...	91	91	...	91	...	...	...	93	93	0.90
D198	49	49	54	46	60	44	48	48	51	51	58	0.51
DCNA	0	1	0	0	[2]	0	0	3	[2]	1	0	0.01
Dexon	87	90	91	89	...	89	81	85	91	87	...	0.88
	1	1	7	6	...	3	...	0	0	4	...	0.03
Dichlone	3	1	3	3	3	2	1	2	3	1	3	0.02
Disulfoton	2	2	1	0	...	3	1	0	1	2	2	0.01
Dodine	0	0	0	0	0	0	0	0	0	0	0	0.00
Dyrene	...	[1]	...	...	[1]	[9]	...	[0]	[0]	1	1	[0.03]
E-275	14	7	...	15	10	9	...	7	5	8	6	0.09
	3	ND	0	ND	ND	ND	2	ND	ND	ND	ND	[0.02]
Ferbam	...	95	...	...	...	95	83	...	[97]	94	...	0.93
	62	63	61	61	[65]	77	64	62	65	65	63	0.64
	34	35	ND	33	42	...	34	ND	ND	ND	ND	0.36
	0	0	ND	ND	[0]	0	12	ND	0	0	0	0.00
Formetanate-HCl	[66]	...	...	[84]	...	[88]	...	[85]	...	72	76	[0.79]
	[15]	...	...	7	[4]	[5]	...	[10]	...	[5]	10	[0.07]
Formparanate-HCl	72	78	83	78	78	85	...	83	72	75	78	0.78
	...	...	[16]	...	15	18	...	9	[6]	...	...	0.13
Hexachlorophene	1	2	1	1	4	1	1	1	1	2	2	0.01
Karathane	...	...	...	...	[26]	...	...	...	[25]	...	...	
	12	14	13	16	...	12	8	18	10	17	10	0.13
	1	ND	ND	ND	5	ND	ND	2	[0]	4	1	0.02
Maneb	31	28	[25]	[60]	43	29	[45]	24	31	41	29	0.33
	ND	0	[14]	16	ND	ND	28	ND	[13]	6	ND	0.00
				[0]			3		1			
Morestan	0	0	0	0	0	0	1	0	0	0	0	0.00
Nabam	...	...	...	...	[94]	...	...	...	[100]	99	...	[0.98]
	[78]	...	...	...	...	...	[75]	...	...	75	81	0.77
	29	...	...	...	33	...	34	30	27	31	39	0.32
	3	...	0	0	5	...	3	2	0	3	1	0.02
Oxycarboxin	75	...	...	[62]	73	71	[0]	...	[71]	75	73	0.73
Panogen 15	82	...	...	...	...	...	...	...	88	83	[87]	0.85
	58	52	...	...	...	57	...	47	55	62	61	0.56
	35	ND	17	35	9	ND	36	ND	ND	ND	[32]	0.35
PCNB	[0]	0	...	0	...	1	2	...	[0]	[1]	[0]	0.00
PCP	36	38	38	39	48	39	39	43	53	43	43	0.42
PCP-(Na)	45	44	44	43	54	45	46	45	57	50	45	0.47
Phenmedipham	...	...	21	...	15	...	...	...	...	...	14	[0.17]
	...	...	...	1	[0]	...	...	[4]	...	[3]	...	[0.02]
Promecarb	...	...	...	43	32	36	[43]	...	[27]	[31]	36	[0.34]
	[0]	...	[3]	[0]	2	0	[6]	...	[0]	1	1	[0.01]
TCNA	0	0	0	2	...	0	1	1	2	0	0	0.00
Terrazole	...	0	...	0	0	...	[0]	0	2	0	0	0.00
Thiram	...	[98]	...	94	[93]	...	...	...	...	...	...	[0.94]
	84	82	59	64	55	80	64	80	82	81	74	0.73
	15	ND	ND	27	35	ND	ND	ND	ND	14	8	[0.13]
	0	0	0	0	16	ND	0	0	0	0	ND	0.00
Zineb	...	...	...	...	[9]	9	...	...	...	...	...	
	0	2	0	0	2	ND	1	1	0	0	0	0.01

Table 2. (continued)

Ziram	...	95	...	93	...	91	...	...	98	...	...	[0.94]
	74	57	59	58	75	66	58	60	76	49	51	0.62
	36	ND	ND	ND	ND	30	ND	ND	52	33	33	0.33
	ND	[19]	16	ND	ND	ND	ND	ND	38	ND	ND	[0.15]
	0	0	0	0	ND	0	0	ND	0	0	0	0.00

[a]Nonfungicides are: disulfoton (insecticide/nematicide), formetanate (acaricide), formparanate (insecticide), phenmedipham (herbicide), and promecarb (insecticide).

[b]Average (sometimes including those of degradation products) based on ≥4 individual determinations. Bracketed value is uncertain, usually because of low sensitivity. Dash indicates nonsensitivity of a bioassay. ND indicated component R_f was not determined.

[c]Abbreviations for organisms are: *Af, Aspergillus fumigatus; Dz, Diplodia zeae; Fm1* and *Fm4, Fusarium moniliforme,* isolates #125 and #412; *Fr, F. roseum; Hs, Helminthosporium sativum; Pc, Penicillium chrysogenum; Pr, P. rugulosum; Rs, Rhizoctonia solani; Tv, Trichoderma viride;* and *Cs, Chlorella sorokiniana.*

[d]Mean based on individual averages for each organism.

[e]Methylmercuric 2,3-dihydroxypropylmercaptide (0.88) and methylmercuric acetate (0.07); the second component was present in most bioassays (though measured with one organism).

Spraying soil with a warm fungi-nutrient agar combination was adopted since it permitted more uniform one-step application of the test organism with better growth characteristics than was achieved without the agar. Figure 1 shows the soil appearance 2 days after spraying a plate with *F. moniliforme* (#412) by this technique.

The soil TLC procedure ultimately adopted was to apply 100 μg each of four compounds per soil plate, replicating in varying spotting sequence on four plates. All 38 compounds were bioassayed simultaneously with one organism. Many additional repetitions were run for most compound/organism combinations and all data were used in the average R_f values reported in Table 2.

Before discussing mobility characteristics of individual compounds, it should be noted that *C. sorokiniana* and the widely distributed (8) soil fungus *T. viride* were the organisms sensitive to the greatest number of fungicides. Compounds inhibitory to all assay organisms were ACNQ, Ceresan L, Ceresan M, D198, dichlone, E-275, ferbam, hexachlorophene, maneb, Morestan, Panogen 15, pentachlorophenol (and PCP-Na), thiram, zineb, and ziram. These include all the organic mercurials and most of the dithiocarbamate and quinone fungicides tested.

The data in Table 2 represent R_f values corrected for diffusion occurring during the bioassay. The increased radius was measured in the upper, nonleached spot. However, subtraction of all or part of this distance from a R_f value depended on the shape of the fungicide zone of inhibition; i.e., compounds exhibiting a narrow, sharply defined front presumably needed no such diffusion correction.

Table 2 is most easily discussed by comparison of chemical classes, and this is facilitated by their grouping in Fig. 2. The sketches are based on the overall mean R_f's and the best qualitative estimate of movement patterns of both parent compound and (in some cases) other fungitoxic components. Since 11 organisms and many sample repetitions were the basis for Fig. 2, we believe it accurately reflects fungicide movement in Hagerstown soil TLC plates.

—1) Aliphatic and organophosphate compounds.—Dodine, an aliphatic fungicide, and disulfoton, an organophosphate insecticide/nematicide, offer an interesting comparison of two basically immobile compounds. Dodine is an organic cation and is thus strongly absorbed to soil because of the soil's cation-exchange capacity. Its immobility is identical with the behavior of the herbicides diquat and paraquat, also organic cations (21). Disulfoton clearly diffuses around the point of application, which probably improves its efficacy in soils. These results conform to the very slight mobility of disulfoton observed in columns of the same Hagerstown soil (13).

—2) Antibiotics.—Three antibiotics constituted one group of fungicides. Cycloheximide and its oxime derivative were equally mobile, but the oxime was less toxic to fungi (Table 2). Thus the decreased tailing of cycloheximide oxime, as compared with cycloheximide, may reflect only a less sensitive bioassay, not the absence of the chemical. E-275, or stendomycin salicylate, is only slightly mobile. This seems reasonable, inasmuch as this mixture of closely related peptides contains a single, strong cationic center in each molecule (2); it would therefore be retained by soil by both ionic bonding and the weaker van der Waals forces. In evaluating whether cycloheximide or any other mobile pesticide might pollute groundwater, one must consider the inherent persistence of the chemical in soils. With cycloheximide, the half-life is very short.

—3) Heterocyclic nitrogen compounds.—Four fungicides were grouped as "heterocyclic nitrogen" compounds (Fig. 2). Dyrene, Morestan, and Terrazole are definitely immobile. Helling (15) earlier reported Morestan's R_f as 0.02 in Hagerstown soil TLC plates, based on autoradiography. No previously published information was found on mobilities of the others. Captan differed significantly in that it leached to a moderate extent [mobility class 3, by prior definition (21)]. The strong origin spot always present with captan is entirely consistent with its low water solubility (≤10 ppm); i.e., the maximum amount of captan that could dissolve during leaching is only 1.0 - 1.5% of that applied, based on the volume of water crossing the origin (14), the sample size, and captan's solubility. These observations seem consistent with those of Munnecke (28), who also found that while most of the captan remained in the upper 2.5-cm layer of a 10-cm column, it did penetrate to the

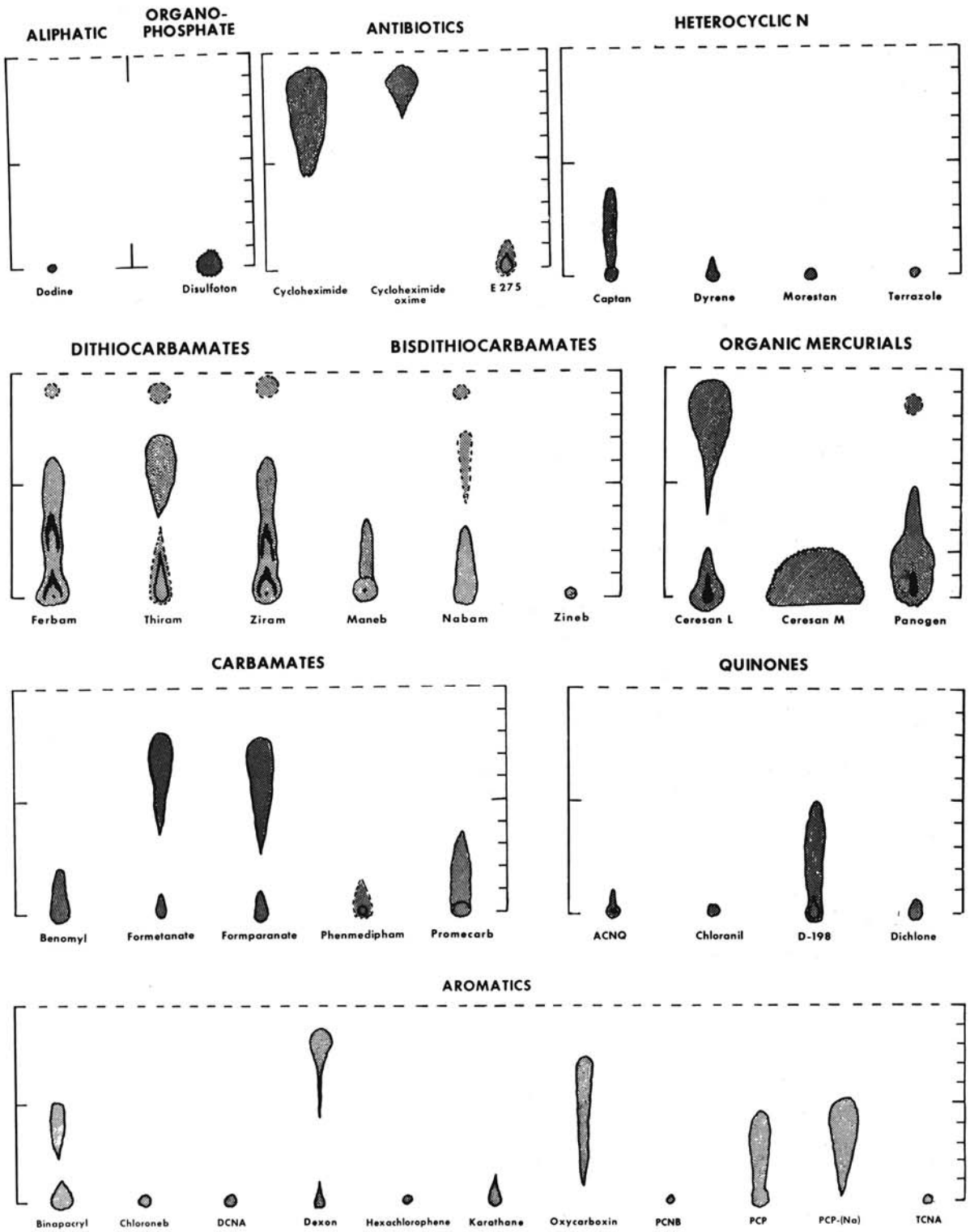

Fig. 2. Movement of 33 fungicides, three insecticides, one acaricide, and one herbicide in Hagerstown silty clay loam TLC plates. Patterns of movement are sketches based on bioassay visualization using 10 fungi and one alga, with at least four repetitions per organism. Zones within dashed lines are uncertain and often seen by growth stimulation only.

lowest quarter in Oakley loamy sand and in admixtures with up to 75% Yolo clay loam. His leaching volume was 1.5 × field moisture capacity. Although data from our soil are not directly comparable, this amount of water would have produced an apparent R_f of 0.59 in Hagerstown.

—4) Dithiocarbamates.—The dithiocarbamates constitute an important category of fungicides in use since the 1930's. The movement of three representative dimethyldithiocarbamates (ferbam, thiram, and ziram) and three ethylenebis(dithiocarbamate)s (maneb, nabam, and zineb) was generally characterized by complex patterns of both fungitoxic and stimulatory zones (Fig. 2). Because these patterns are similar among the dimethyldithiocarbamates, we speculate that their degradation products (presumably in the original samples) are identical. The dithiocarbamates do yield many by-products, often fungitoxic (27, 32); unfortunately, the scope of our experiments did not permit us to isolate and identify the unknown components.

Solubility of nabam is high (30%), and thus does not inherently limit its movement. The intermediate movement to R_f 0.32 is assumed to be the parent compound, detectable by seven test organisms (Table 2). Other apparent breakdown products (R_f 0.98 and 0.77) were less often detectable, except for an origin spot. Nabam, maneb, and zineb were examined in a separate investigation of ethylenethiourea (ETU) mobility in soils (20), and the results of related autoradiography experiments follow in a separate section. Bioassay results indicated that zineb is immobile and seemingly free of bioactive contaminants. Maneb has both a strong origin spot and limited streaking. Partly on the basis of zineb's immobility and partly because the strongest inhibition always occurred at the origin, we suspect that the other three heavy metal salts are also immobile in soil. The streaking of maneb, virtually identical with that of nabam, may reflect some dissociation of the metal complex and movement of the resultant free ethylenebis(dithiocarbamate). That species would be identical with dissociated nabam.

In a comparative study of 13 fungicides, Munnecke (28) found that nabam was mobile in a loamy sand, and somewhat less so in a clay loam. If movement is considered only through the zone showing major fungicidal activity (minor inhibition occurred below this depth), then his data show nabam to leach to ca. R_f 0.5 in Yolo clay loam. In a leaching test (28) of fungicides penetrating 10-cm columns of U.C. mix, an artificial soil-peat moss mixture, the following distribution was observed: nabam, 0-10 cm; thiram, 0-7.5 cm; captan, 0-7.5 cm; and zineb, 0-2.5 cm. Zineb and ferbam were both reportedly immobile. Kendrick and Middleton (24) using a soil drench test, presumed to reflect movement in soil, found that nabam and, to a lesser extent, thiram were effective (i.e., mobile), but PCNB and captan were not (i.e., immobile). The previously cited studies (24, 28) may be contradicted by other results in which bioassays showed 99% of added nabam to remain in the upper 5 cm of a sandy loam (5), and thiram, in the upper 2.5 cm (30). All such interpretations must be made judiciously, though, because of species dependence and (as is clear in Fig. 2) other bioactive contaminants or transformation products.

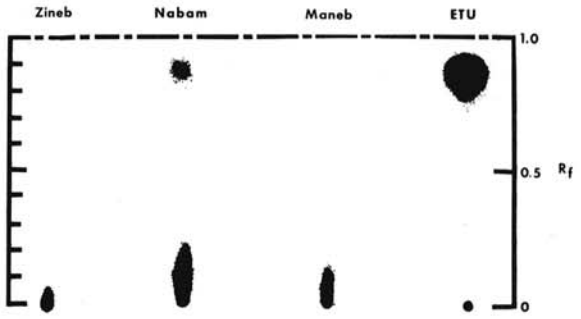

Fig. 3. Movement of ^{14}C-labeled ethylenethiourea (ETU) and fungicides maneb, nabam, and zineb in a Hagerstown silty clay loam TLC plate. Visualization is by autoradiography. Sample size of ETU and its precursors was 1 μg.

TABLE 3. Mobility of nabam, maneb, and zineb fungicides in soil TLC plates

Soil	Organic matter content, (%)	Average[a] R_f of		
		Nabam	Maneb	Zineb
Norfolk sandy loam	0.14	(0.98) 0.48 0	(0.96) 0.42 0	0.15 0
Lakeland sandy loam	0.90	(0.96) 0	(0.83) 0	0.16 0
Hagerstown silty clay loam	2.50	(0.94) 0.22 0	(0.91) 0.17 0	0.10 0
Barnes clay loam	6.90	(0.80) 0.14 0	(0.73) 0.10 0	0.08 0
Celeryville muck	90.4	(0.53) 0	(0.51) 0	0

[a]Average of four repetitions, based on autoradiography visualization. Sample size was 1 μg. Values of some impurities shown in parentheses.

—5) Organic mercurials.—The fungicides that seemed most toxic, and thus easiest to detect on soil TLC, were the organic mercurials (Fig. 2). Ceresan L and Panogen 15 were formulated with a red dye, for use in seed treatment, and this dye was only slightly mobile, as designated by the darker zones. Ceresan L contains two active ingredients, methylmercuric acetate (MMA) and a methylmercuric mercaptide. Their leaching characteristics were completely different. Although we found no direct evidence indicating which component is the mobile one, it seemed reasonable that it was methylmercuric 2,3-dihydroxypropylmercaptide, which should be mainly undissociated in solution. Methylmercuric acetate, by contrast, has a relatively high ionization constant (37); the resulting methylmercury cation, the toxic portion of organic mercurials (39), should adsorb to the soil by ion exchange and therefore be rather immobile.

In this respect, it may resemble phenylmercuric acetate (PMA), which largely remained in the upper layer of some Japanese field soils (1). However, mercury residue distribution was dependent on soil mineralogy; soils that immobilized mercury were montmorillonitic, whereas those in which leaching occurred were kaolinitic. These observations were confirmed in the laboratory, where PMA was irreversibly adsorbed to montmorillonite and only slightly bonded to kaolinite (23). The clay in our Hagerstown soil is vermiculite/kaolinite, the soil cation-exchange capacity is 14.7 meq/100 g; although this value is lower than that expected for a montmorillonitic soil of comparable clay and organic matter contents, it is nonetheless large enough to retard, we assumed, movement of MMA or PMA.

We tested this theory by leaching 5, 10, 50, and 100 μg applications of MMA, PMA, and Ceresan L (calculated as MMA) on Hagerstown plates. Bioassays (*Chlorella* and *T. viride*) confirmed that both MMA and PMA were immobile, though PMA diffused less than MMA. The immobile component of Ceresan L behaved identically with MMA, which also proves that the mobile component is methylmercuric 2,3-dihydroxypropylmercaptide.

Ceresan M is a compound not inherently leachable but which exhibits significant short-range diffusion. Its diffusion-corrected R_f of 0.05 places Ceresan M in mobility class I (21), though it certainly is differentiated from ACNQ, DCNA, dyrene, and other immobile pesticides.

Panogen 15, the third organic mercurial, again was characterized by lateral diffusion, which probably greatly aids its effectiveness. Vapor phase movement is likely important, for inhibition often occurred in a broad zone leeward of the forced air source used to dry spots during application. This observation is consistent with soil column studies (29). The other pesticides we studied showed no such effect. An unknown mobile component was detected, sometimes by stimulation only, by five organisms (Table 2). Cyano(methylmercuri)guanidine itself has intermediate mobility, though its unusual pattern suggests two bioactive components.

—6) Carbamates.—Benomyl, a well-known systemic carbamate fungicide, showed only limited movement in soil (Fig. 2, Table 2). This is consistent with leaching behavior in intact field soil cores (34). Although Pitblado and Edgington (34) assumed movement was actually due to benomyl's fungitoxic degradation product, methyl 2-benzimidazolecarbamate (MBC), we believe our data reflect movement of benomyl *per se,* since leaching was complete within ca. 2 h. Subsequent hydrolysis to MBC during the bioassay is probable.

The remaining carbamates were difficult to visualize by the fungal bioassay. Formetanate, an experimental acaricide, and formparanate, an experimental insecticide, are similar chemically and in their mobility characteristics. Both apparently contain an immobile impurity or breakdown product. Formparanate was more toxic than formetanate and this is perhaps related to its higher solubility. Phenmedipham, a new herbicide, appears rather immobile. Promecarb, an insecticide, was also poorly visualized by fungal or algal bioassays; the origin and the streak may be separate entities.

—7) Quinones.—One of the quinones (Fig. 2), ACNQ, has been used as a paddy rice herbicide. In soil columns, it remained largely in the upper layer, with only a trace leaching down (12). Our results confirm this finding. Dichlone was immobile in soil columns (28) and was immobile and/or inactivated in a soil drench test (41); chloranil and dichlone were both predicted by Goring (10) to be immobile in soils. Again, we found both to be immobile. ACNQ, chloranil, and dichlone were all readily detectable by *Chlorella* as well as by fungi, which corresponds to an earlier report that these quinones are algicidal (42). No information was found on D198. Chemically, it differs from the others because one carbonyl oxygen has reacted to form an oxime derivative. This substantial increase in mobility apparently produced by derivatization may indicate a mechanism for increasing the soil penetration of certain fungicides.

—8) Aromatics.—The 11 fungicides in the final group are all aromatics. One is unique, Dexon, because it is an organic anion. Thus, by the same reasoning that dodine is attracted strongly to soil, we anticipated Dexon to be repelled by most soil clays and therefore to be rather mobile in soil. It was quite mobile, and was accompanied by a slightly fungitoxic immobile spot which may be a photodegradation product. To reduce rapid detoxication, we usually leached soil TLC plates in semi-darkness when they contained Dexon. Raabe and Hurlimann (35) reported that ca. 90% of Dexon in a soil drench remained within 5 cm of the surface in a potting soil mix. However, our calculations suggest that simply too little water was added to have transported Dexon further into the soil column; Raabe and Hurlimann did note that additional water increased the penetration. Another factor that probably retards Dexon movement is sorption to soil organic matter.

Aromatic fungicides that were immobile include chloroneb, DCNA, hexachlorophene, PCNB, and TCNA. Rhodes et al. (36) showed the same pattern of chloroneb movement, by soil TLC, as we found. DCNA is reportedly strongly adsorbed to soil (11), so its immobility is expected.

Pentachlorophenol, its sodium salt, and oxycarboxin were all moderately mobile. The slight difference between the two PCP formulations probably results from the higher solubility of PCP-(Na), which is completely leached from the origin. This biocide also effectively penetrated soil in Zentmyer's drench test (41).

Finally, two similar phenolic esters are compared, binapacryl and Karathane. Although the second shows only one spot, consistent with a single compound, binapacryl apparently had two components. Some hydrolyzed product may have been present. Binapacryl's more mobile component may be the parent phenol, which (at least for Karathane) is known to be fungitoxic. By analogy with other pesticides, the parent ester is unlikely to be very mobile and therefore is probably shown by the R_f 0.03 spot. Restricted mobility of Karathane is suggested by soil drench tests (30, 41).

Leaching experiments (autoradiogram).—We compared leaching patterns of a few radioactive fungicides with bioassay results from their nonlabeled counterparts. Morestan, reported earlier, was immobile by both autoradiographic (15) and bioassay visualization.

[^{3}H]Phenmedipham was very difficult to detect, but extremely faint film darkening occurred only at the origin, which suggests the herbicide is not mobile.

Because the dithiocarbamate fungicides nabam, maneb, and zineb produce ETU, a carcinogen and goitrogen, a recent study in our laboratory dealt with ETU's movement and adsorption (20). Both precursors and ETU were ^{14}C-labeled, affording the typical chromatogram shown in Fig. 3. The bioassays of Fig. 2 depicted any component in sufficient quantity to inhibit or stimulate fungal or algal growth, or any component rapidly converted in situ to other substances possessing such biological activity. The radioactive samples show any sufficiently abundant labeled component, regardless of bioactivity. Impurities derived from the synthesis may well differ, since synthetic routes in radiochemistry are often dissimilar from those of commercial pesticide production. [^{14}C]Nabam and [^{14}C]maneb, especially, contained numerous impurities, some of which continued to form during extraction and rechromatography. Nabam's radioactivity was concd at R_f 0, streaking to R_f 0.22. A significant impurity which chromatographically resembled ETU occurred at R_f 0.94. Maneb also had a strong origin and was slightly mobile, with very faint movement to what is presumably ETU. Zineb showed only a strong origin and short, faint streaking. Autoradiograms differ from the bioassays mainly in that bioassays showed somewhat more movement of maneb and nabam samples, while showing less for zineb.

Table 3 is a summary of movement of nabam, maneb, and zineb in five soils. Additional soil descriptions and ETU mobility data are presented elsewhere (20). As soil organic matter increased, mobilities decreased; e.g., the R_f 0.48 component of nabam in Norfolk sandy loam was completely immobile in Celeryville muck. Lakeland sandy loam, common in Eastern United States, is coarse textured and rather nonrestrictive of pesticide movement (16). Streaking of nabam and maneb was so extensive that separate components were not distinguishable, even though seen in the other mineral soils. The zone reported in parentheses always resembled ETU and was much stronger in nabam than in maneb. From data of Tables 2 and 3, the probable order of mobility is nabam > maneb > zineb.

The fundamental trends of mobility derived by autoradiography seem to agree with bioassay data. These, in turn, are in general accord with published observations of fungicide movement, where such data are available. It is for this reason, and because herbicide mobility trends have also been in similar accord with field data, that we consider soil TLC results to be useful indications of pesticide leaching behavior. We recognize the uniformity of the thin soil layer as contrasted with the far more heterogeneous soil profile. However, any laboratory method, even the use of lysimeters, is as yet a faulty approximation of field behavior. Soil TLC has a unique role in its application for examining many compounds in natural or modified soils, under easily standardized conditions.

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