

2-naphthol, 2-hydroxy-1-naphthoic acid, and bis(2-hydroxy-1-naphthyl) ketone, all produce colors with the same absorption maximum as Squoxin. From the results presented in Table III, we conclude that the latter compounds are not produced in significant amounts during the field use of Squoxin.

Final Procedure for Colorimetric Analysis. A calibration curve is prepared from standards containing 2 to 10 μg of Squoxin in 1 ml of ethanol and 3.2 ml of pH 8 buffer and 0.8 ml of Diazo Blue B reagent are added. The absorbance at 552 nm is measured within 10 min. Data for a standard curve showing the precision of this step are presented in Table V.

The 1500-ml water sample in a 2000-ml separatory funnel is acidified with 1 ml of 6 N HCl and 50 ml of 2-propanol and 25 ml of CCl_4 are added. The mixture is shaken for 2 min, the layers allowed to separate, and the lower layer removed. The aqueous layer is re-extracted with 15- and 10-ml portions of the solvent and the extracts combined and filtered if necessary. A suitable aliquot (2 to 10 μg of Squoxin) is taken and the CCl_4 removed by evaporation. The residue is dissolved in 1 ml of ethanol, and 3.2 ml of

pH 8 buffer added followed by 0.8 ml of the Diazo Blue B reagent. The absorbance is measured at 552 nm within 10 min.

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Adsorption, Mobility, and Persistence of Thiabendazole and Methyl 2-Benzimidazolecarbamate in Soils

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Adsorption of thiabendazole (TBZ) and 2-benzimidazolecarbamate methyl ester (MBC) to three soils was studied. With increasing acidity of the soil suspensions an increase in the adsorption of these fungicides to the soil occurred. Results indicated ionization of benzimidazole fungicides at lower pH values, and adsorption of these ionized molecules on the soil. Thiabendazole was adsorbed to the soil in much larger quantities than MBC, and therefore at equilibrium TBZ was detected in the free soil solution in much smaller

concentrations. The mobility of TBZ in the soil was also much smaller than that of MBC. The persistence of these fungicides in soil samples, incubated at 25° in small glass vials, was also examined. Nine months after adsorption of these fungicides to the soil, 85–95% of the applied TBZ and 65–75% of the applied MBC were recovered from air dried soils. However, in moist soil after 9 months of incubation only 75–90% of the applied TBZ and 20–30% of the applied MBC were recovered.

Previous experiments have shown that uptake of systemic benzimidazole fungicides by plants from the soil is very inefficient. Erwin (1973) stated that control of vascular wilt diseases required extremely large dosages and was too limited to be of practical control for deep-rooted crops. Presumably this was the result of tight adsorption of these residues to soil (Baude et al., 1974). It has been shown that more benomyl was taken up from sandy soils than from clay soils (Fuchs et al., 1970), and that the rate of uptake of benomyl by plants was increased when they were grown on soils with low organic matter content, and with higher pH (Schreiber et al., 1971). The relative long term persistence of benzimidazole fungicides in the soil and their low water solubility and movement in soil have been demonstrated by Hine et al. (1969), Baude et al. (1974), and Rhodes et al. (1974).

Since adsorption of benzimidazole derivatives by the active surfaces of the soil may govern the effectiveness of the fungicide and may also affect their persistence and leaching properties, a study was undertaken to further examine these parameters. A mechanism of adsorption of benzimidazole derivatives on mineral clays has previously been suggested by Aharonson and Kafkafi (1975). In this investigation, further studies were conducted on adsorption mechanism, mobility, and persistence of these fungicides in various soils.

EXPERIMENTAL SECTION

Materials. *Soils.* Three soils having substantial differences in levels of clay, CaCO_3 , organic matter, and cation capacity were used in this study (Table I).

Chemicals. Analytical grade and technical 2-benzimidazolecarbamate methyl ester (MBC) as well as analytical grade and technical thiabendazole (TBZ) were kindly provided by E. I. DuPont de Nemours & Co. Inc., E. Merck, and Agan Chemicals.

Methods. *Analytical Methods; Extraction from Soil and Determination of TBZ, MBC, and 2-Aminobenzimidazole.* Ten-gram subsamples, taken from air-dried and

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Table I. Properties of the Soils Used in this Study^a

Soil ^b type	Soil fraction, % of total					CaCO ₃ , %	Cation exchange capacity	Organic matter	pH
	Coarse sand	Sand	Silt	Clay					
Hamra	7.5	75.2	3.6	13.1		2.2	13.8	0.8	7.8
Loess	2.2	53.3	34.7	9.7		15.4	15.7	0.9	7.9
Hydromorphic grumusol	8.9	38.3	21.3	31.4		4.3	33.4	1.2	7.9

^a The authors express their thanks to Dr. H. Koyumdjisky for her help in defining the soil properties. ^b The hamra and the hydromorphic grumusol soils belong to the same catena in Bet Dagan; the loess soil was sampled at the Gilat Experiment Farm near Be'er Sheva'.

mixed soil, were shaken for 30 min with 200 ml of 1 *N* NaOH. The aqueous suspension was extracted three times with 150 ml of ethyl acetate. The combined ethyl acetate phases, after concentration at 40° in a rotary evaporator to 300 ml, were extracted three times with 0.1 *N* HCl (100, 50, and 50 ml). To the combined aqueous washings 25 ml of 3 *N* NaOH was added before extraction with 150 ml of ethyl acetate. Two more extractions of the aqueous washings were performed with the addition of 5 ml of 3 *N* NaOH and 50 ml of ethyl acetate. The ethyl acetate phases were combined, dried over anhydrous Na₂SO₄, and evaporated to dryness in a rotavapor. The residues were redissolved in 12.5 ml of ethyl acetate and 37.5 ml of petroleum ether was added. The solution was transferred to an alumina-magnesium oxide-Celite chromatographic column. The chromatography procedure was previously described in detail by Aharonson and Ben-Aziz (1973), with the exception of the separation and determination of 2-AB. TBZ was eluted from the column with 150 ml of ethyl acetate, MBC was eluted with 150 ml of 25% absolute ethanol in ethyl acetate, and the more polar compound 2-aminobenzimidazole was eluted with 100 ml of 98% ethanol. Each fraction was evaporated to dryness with a rotavapor, redissolved in 10 ml of ethanol, and subjected to analyses with a fluorescent spectrophotometer. Thiabendazole was determined at an excitation wavelength of 305 nm and an emission of 345 nm, MBC at an excitation of 282 nm and an emission of 307 nm, and 2-AB at an excitation of 280 nm and an emission of 317 nm.

Recovery of added amounts of TBZ from the soil was 80–85%, of MBC 70–75%, and of 2-AB 60%. Limits of detection were 0.02 ppm for TBZ, 0.05–0.1 ppm for MBC, and 0.03 ppm for 2-AB.

The amounts of the two fungicides adsorbed to the soil were not affected by air drying or even by placing soil samples in the oven at 100°.

Analysis of Soil Suspensions. Soil suspensions, obtained from studies to determine adsorption isotherms and equilibrium phenomena, were filtered through Whatman No. 1 filter paper. An aliquot of 20 ml of the clear solution was placed into a 150-ml separatory funnel, and 10 ml of 3 *N* NaOH was added. This basic aqueous phase was then extracted three times with 30 ml of ethyl acetate. TBZ was determined directly from the ethyl acetate phase with a fluorescent spectrophotometer (305-nm excitation, 345-nm emission). For MBC determination, the ethyl acetate phase was evaporated to dryness with a rotavapor; the residue was redissolved in 10 ml of absolute ethanol and analyzed with a fluorescent spectrophotometer (282-nm excitation and 307-nm emission).

In this study the following four experiments were conducted.

(1) **Determination of Adsorption Isotherms.** Air-dried soils (25 or 50 g), after passing through a 0.5-mm sieve, were placed in 100- or 250-ml erlenmeyers. Analytical grade fungicide (recrystallized) dissolved in water was

added to the soil to achieve concentrations of 1–10 ppm, but never above its water solubility. The final volume of water in the flasks amounted to 150 ml. The flasks were then covered with aluminum foil, sealed with parafilm, and shaken for 8 hr at ambient temperature (20 ± 1°). The soil-water suspensions were filtered through Whatman No. 1 filter paper and the clear solutions were used to determine the amounts of fungicide remaining in the water. In several cases the soil precipitate was also analyzed for adsorbed fungicide. Samples consisting of soil and water at a ratio of 2:1 were passed under suction through fritted disk funnels. All data presented herein are results of at least duplicate analyses of two separate experiments. The pH of the suspensions was changed by the addition of either 0.1 *N* HCl or 0.1 *N* NaOH, and determined with a glass electrode pH meter.

(2) **Release of Fungicide from Soils into Water.** Air-dried soil was treated with an ethyl acetate solution (0.1–1.0 ml/25 g of soil) of the fungicide. The soil was air dried and 10-g aliquots of the treated soil were subjected to analyses as described above. Twenty-five gram aliquots were placed into each of a series of 250-ml erlenmeyer flasks. Twenty-four to forty-eight hours later, 25 ml of water was added to each 25-g soil sample followed by continuous shaking of the flasks for 8 hr. After that the soil suspensions were filtered and analyzed as described above.

(3) **Movement of the Fungicides through Soil Columns with Water.** Air-dried and sieved loess soil was packed on top of a glass wool plug in a 40 × 4.5 cm PVC column, resulting in a soil column height of 22 cm. The fungicides adsorbed from ethyl acetate to a 50-g soil sample were placed on top of the 22-cm soil column. A 2-cm layer of untreated soil was then placed on top of the fungicide treated soil. The amounts of fungicides placed on the columns were 4500 µg of TBZ and 2200 µg of MBC. The fungicides were eluted with 1700 ml of H₂O (equivalent to 1000 mm of rain), an experiment that took 12 days. To determine the amount of fungicide that had moved through the soil with water, the eluted fractions were collected and analyzed as described. At the end of the experiment the moist soil was removed from the column, cut into ten 2.2-cm tall sections, air dried, and thoroughly mixed. Duplicate 10-g subsamples were then analyzed for the remaining fungicide.

(4) **Persistence of TBZ and MBC in the Soil.** Water solutions of TBZ (8.0 ppm) and of MBC (4.5 ppm) were prepared. Five hundred milliliters of the solution was percolated through different 500-g portions of air-dried soil that previously had been placed into a plastic funnel. After termination of the water percolation, the respective soils were air dried and mixed. Ten-gram subsamples were utilized for extraction and analysis as described, indicating concentrations of 6 ppm of TBZ and of 2–3 ppm of MBC in the soils. For incubation purposes 10- and 25-g subsamples of soil were placed into glass vials. One series of soil samples was kept dry, a second one was moistened to field capacity, and a third one was held under anaerobic conditions by

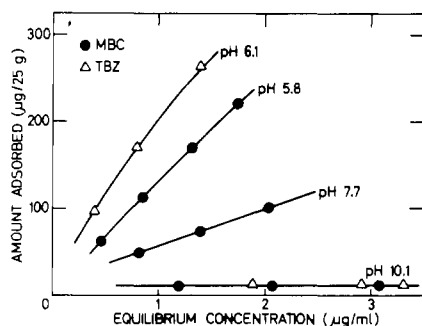
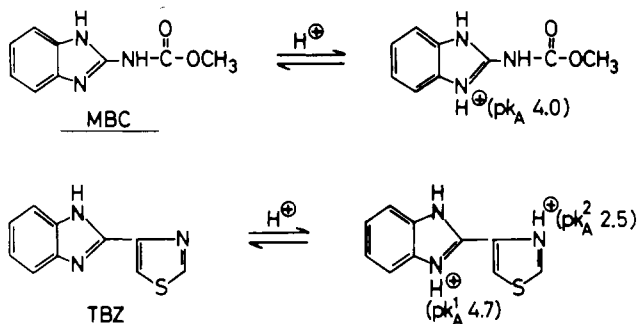


Figure 1. The effect of pH on the adsorption of TBZ and MBC by loess soil.

adding sufficient water to produce a 1-cm layer of water above the soil. This water level was kept constant by adding water as required. The three series were then incubated at 25°. At zero time and at 3, 6, and 9 months after the soil treatment duplicate samples of each series were extracted and analyzed for the remaining fungicides as described above.

RESULTS AND DISCUSSION

Adsorption isotherms for TBZ and MBC on loess soil at different pH values are shown in Figure 1. The amount of fungicide adsorbed by the soil was increased at lower pH values, a finding similar to that obtained from adsorption isotherms of benzimidazole derivatives on mineral clays by Aharonson and Kafkafi (1975). The same authors suggested that the mechanism of adsorption on clay surfaces is due to protonation of these basic organic molecules and therefore is dependent upon the acidity of the clay surfaces. Thus, the pH dependence of the adsorption of benzimidazole derivatives by soils may also be due to protonation of the molecules on the soil surface.



Adsorption isotherms for TBZ and MBC on three different soils are shown in Figure 2. Thiabendazole was adsorbed in much larger quantities than was MBC. This was again similar to the results obtained from adsorption studies on clay minerals. The adsorptive capacity of montmorillonite for TBZ (at equilibrium at pH 7.6 and in 0.01 M CaCl₂) was 25 times greater than that for MBC (Aharonson and Kafkafi, 1975). This ratio was found to be much smaller in the soil—approximately 1:3 to 1:4. If adsorption to soil is mainly by protonation on clay surfaces, the largest amount should be adsorbed to the hydromorphic grumusol soil since its cation exchange capacity (Table I) is twice as high as that of the two other soils. In fact, the amount of TBZ adsorbed to the hydromorphic grumusol soil was almost twice as much as that adsorbed to the hamra and loess soils. This ratio was somewhat different for MBC. On the other hand the organic matter content of the hydromorphic grumusol soil is also higher than that of the two other soils, and the ionized molecules could also be adsorbed by the organic fraction of the soil. The role of organ-

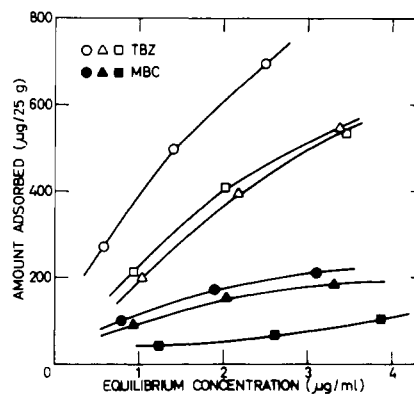


Figure 2. Adsorption isotherms for TBZ and MBC on three different soils: (O, ●) hydromorphic grumusol; (Δ, ▲) loess; (□, ■) hamra.

Table II. Concentration of TBZ and MBC in the Soil-Water at Equilibrium and the Relation to Their Initial Concentrations in the Soil

Quantity of fungicide adsorbed to soil, ^a ppm	Concn of TBZ in the soil-water at equilibrium, ppm		Concn of MBC in the soil-water at equilibrium, ppm	
	Loess	Hydromorphic grumusol	Loess	Hydromorphic grumusol
30	2.3			
24	1.8		9.5	
16	1.2	0.3	5.8	5.5
8	0.5	0.15	2.4	2.2
4	0.25	0.07	1.2	1.0
2	0.1		0.5	
1	0.05		0.25	

^a The fungicides were adsorbed to 25 g of soil in ethyl acetate; the soil was air dried and shaken for 8 hr with 25 ml of water.

Table III. Concentration of TBZ and MBC in the Water at Equilibrium in Various Water-Soil Ratios

Fungicide	Quantity of fungicide adsorbed to the soil, ppm	Soil	Ratio water: soil	Concn in the water at equilibrium, ppm
TBZ	8	Loess	1:2	0.44
		Loess	1:1	0.45
		Loess	2:1	0.52
		Loess	4:1	0.52
		Loess	8:1	0.41
TBZ	5	Hydromorphic grumusol	1:2	0.15
		Hydromorphic grumusol	1:1	0.12
		Hydromorphic grumusol	2:1	0.18
MBC	4	Loess	2:1	0.8
		Loess	3:1	0.7
		Loess	4:1	0.6

^a Fungicides applied to 50 g of soil in ethyl acetate, air dried, and shaken with water for 8 hr.

Table IV. Elution of MBC from a Loess Soil Column^a

Fraction	Vol of fraction, ^b ml	MBC found, ^c μg
I	265	20
II	200	133
III	312	436
IV	350	517
V	208	213
VI	366	245

^a A total of 2200 μg of MBC was applied to 500 cm^3 of soil; 1700 ml of water was passed through the column. ^b Column volume = volume of water in column which was for loess soil 250 ml. ^c Only trace amounts of MBC were detected in the column.

These findings suggest that TBZ is available in the soil-water at much smaller quantities, but over a longer period of time. The amount of TBZ adsorbed to the soil might be considered as a reservoir of fungicide for the plant since the adsorption is a reversible process and the degradation of the adsorbed fungicide is very slow as shown below.

Results in Table III indicate that with TBZ about 5% of the fungicide adsorbed to the loess soil was found in the water at equilibrium, and less than 5% with the grumusol soil. With MBC and the loess soil, about 15–20% of the adsorbed fungicide was found in the water.

It is also evident that at different soil-water ratios the concentration of the fungicide in the solutions did not change much. The lower ratio of 2 parts of soil to 1 part of

Table V. Effect of Moisture and Time on the Disappearance of Adsorbed TBZ from Three Soils^a

Soil type	Moisture content	TBZ residues, ppm			
		0 ^d day	After 3 months	After 6 months	After 9 months
Hamra	Dry ^b	6.9	6.7	6.7	6.5
	Field capacity (aerobic)	5.6	4.6	4.0	3.9
	Anaerobic ^c	5.6	4.9	3.9	3.5
Loess	Dry	6.8	7.0	6.2	6.1
	Field capacity (aerobic)	5.4	5.0	4.7	4.6
	Anaerobic	5.4	5.0	4.7	3.5
Hydromorphic grumusol	Dry	5.1	4.8	5.1	5.3
	Field capacity (aerobic)	4.7	4.1	3.8	4.2
	Anaerobic	4.7	3.7	3.7	4.2

^a Recrystallized TBZ was adsorbed to the soil. ^b Air-dried soil. ^c One centimeter of water above the soil. ^d Differences in the initial concentration due to different soil batches.

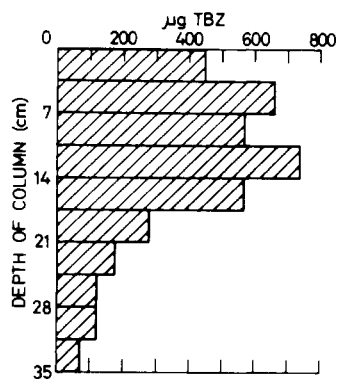


Figure 3. Distribution of TBZ in a loess soil column after 1700 ml of water had percolated through it. TBZ (4500 μg) was applied to a 50-g soil layer placed on top of the soil column.

ic matter in the adsorption of benzimidazole derivatives is still under investigation.

The differences in the adsorption of TBZ and MBC by the same soil, and the differences in the amounts of each fungicide adsorbed on various soils, as obtained by adsorption isotherms, may represent the actual variations in the availability of this fungicide to the plant. This assumption was examined in the following experiment in which TBZ and MBC were adsorbed to the soil, the soil was shaken with water at a ratio of 1:1 by weight, and their concentrations in the soil-water at equilibrium were determined (Table II). It can be seen that the concentration of TBZ in the soil-water amounted to 20% of that of MBC after identical amounts had been applied to loess soil; with the hydromorphic grumusol soil, however, this percentage was only 7–8%.

water (w/w) might represent the real concentration of the fungicide in the soil solution at field conditions.

The differences in adsorption of TBZ and MBC on the soils were also shown in the leaching characteristics of each compound. While TBZ moved through the soil column with water to a distance of 10–20 cm (Figure 3), MBC was eluted from the soil column under the same conditions. The amount of MBC found in the eluted fractions is shown in Table IV. MBC was already detected in the first fraction collected, while TBZ was still in the upper half of the column, after water equivalent to 1000 mm of rain had passed through the column.

In this experiment total recovery of TBZ and MBC from the soil columns, including the amounts extracted from the soil and from the water eluates, amounted to 83 and 77%, respectively, of the applied dosages.

The rate of movement of MBC in the column points to a very low mobility under field conditions, while thiabendazole could be considered as practically immobile in the soil.

Persistence of TBZ and MBC in Incubated Soils. Results obtained with experiments to determine the persistence of TBZ and MBC in three different soils are summarized in Tables V and VI. Thiabendazole was very persistent in air-dried soil. In aerobic and flooded (anaerobic) soils, losses of 10–25% were recorded over a period of 9 months. Results for MBC were somewhat different. In air-dried soils, losses of MBC during 9 months amounted to 25–35% of the total. In the moist soil (aerobic and anaerobic), losses were much higher, amounting to 70–80% of the total, 9 months after treatment.

MBC in the three soils disappeared much faster during the first 4 months than during the last 3 months of the experiment, when almost no change in the level of residues occurred.

Table VI. Effect of Moisture and Time on the Disappearance of Adsorbed MBC from Three Soils^a

Soil type	Moisture content	MBC residues, ppm			2-AB residues, ppm, after 9 months	
		0 ^d day	After 3 months	After 6 months		After 9 months
Hamra	Dry ^b	2.1	1.9	1.8	1.7	0.2
	Field capacity (aerobic)	2.4	0.9	0.6	0.8	0.08
	Anaerobic ^c	2.4	1.5	0.4	0.6	0.06
Loess	Dry	2.8	2.0	2.5	1.8	0.2
	Field capacity (aerobic)	2.2	0.7	0.6	0.8	0.1
	Anaerobic	2.2	0.4	0.5	0.5	0.1
Hydromorphic grumusol	Dry	3.3	2.9	3.3	2.2	0.3
	Field capacity (aerobic)	3.2	1.3	0.9	0.5	0.1
	Anaerobic	3.2	2.1	0.7	0.6	0.1

^a Recrystallized MBC was adsorbed to the soil. ^b Air-dried soil. ^c One centimeter of water above the soil. ^d Differences in the initial concentration due to different soil batches.

A possible degradation product of MBC, 2-aminobenzimidazole, was detected in the MBC-treated soil but in very small quantities. The results obtained in this laboratory test are in close agreement with data reported by Baude et al. (1974) for benomyl in field experiments.

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Absorption, Excretion, and Metabolism of 1,3-Bis(*p*-chlorobenzylideneamino)guanidine Hydrochloride (Robenz Robenidine Hydrochloride) in the Chicken

Jack Zulalian,* David A. Champagne, Richard S. Wayne, and Roger C. Blinn

When chickens were given a single oral dose of robenidine hydrochloride labeled with ¹⁴C in either the α -carbon atom of the *p*-chlorobenzylidene moiety or in the aminoguanidine carbon atom, they excreted 82% of the administered radioactivity within 24 hr. A major portion of this radioactivity corresponded to robenidine. Several major metabolites, retaining only the *p*-chlorobenzylidene-¹⁴C label of robenidine, were found. These were isolated by solvent extraction and purified by column chromatography. Mass spectral analysis of

the diazoethane derivatives of the metabolites indicated that they were mixed conjugates of ornithine and lysine containing *p*-chlorobenzoic acid with either benzoic acid or *p*-hydroxybenzoic acid. Robenidine and a metabolite identified as 3-amino-4-(*p*-chlorobenzylideneamino)-5-(*p*-chlorophenyl)-4*H*-1,2,4-triazole were found in fat, liver, and skin while robenidine and the ornithine and lysine conjugates of the acids were found in liver, kidney, and muscle.

The compound 1,3-bis(*p*-chlorobenzylideneamino)guanidine hydrochloride, the active ingredient in Robenz (registered trademark of American Cyanamid Co.) robenidine hydrochloride medicated premix coccidiostat manufactured by American Cyanamid Company, has been regis-

tered for use as an effective, safe feed additive product for the prevention of coccidiosis in broiler chickens. Robenidine hydrochloride is not related chemically to any of the previously or presently used anticoccidials and, therefore, represents an entirely new structure in the field of coccidiosis control. Robenidine hydrochloride is highly efficacious against the six major species of *Eimeria* that infect chickens (Kantor et al., 1970). It has a dual mode of action (Ryley and Wilson, 1971) in that it arrests the development

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