

ylation or the alkylation reaction constitutes a suitable method for confirmation and detection of hydroxyatrazine residue levels down to approximately 0.5 ppm when present in a soil sample. Although alkylated atrazine and alkylated hydroxyatrazine have overlapping retention times on a Carbowax 20M column (Figure 6), the former will not interfere with the gas chromatographic detection of the latter after column separation through silica gel (Figure 1). The derivation methods developed for hydroxyatrazine are believed to be applicable, with minor modification, to other 2-hydroxy-*s*-triazines.

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Adsorption of Benzimidazole Fungicides on Montmorillonite and Kaolinite Clay Surfaces

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The effect of pH on the adsorption by montmorillonite and kaolinite clays of three benzimidazole derivatives, thiabendazole (TBZ), 2-benzimidazole carbamic acid methyl ester (MBC), and benzimidazole, was investigated. Adsorption isotherms for TBZ and MBC on Ca and Na Wyoming bentonite showed that an increase in the acidity of the suspension resulted in a significant increase in the adsorption of the fungicide to the clay. The shape of the adsorption curves on Ca-bentonite at various pH values resembled simple titration curves with a midpoint 2.5-3.5

pH units above the pK_a of the respective fungicides. The midpoint for TBZ on Na-kaolinite was in the vicinity of its pK_a . The fungicide MBC did not adsorb on kaolinite clay even at pH 2. These findings suggested adsorption on mineral clay surfaces by protonation of these basic organic molecules. Protonation and adsorption were not directly related to the basicity of the molecule. An increase in $CaCl_2$ concentration resulted in a decrease in the adsorption of the fungicides. Both fungicides were adsorbed in aqueous solutions on MgO and not adsorbed on Al_2O_3 .

Benzimidazole derivatives were introduced as systemic fungicides capable of controlling a wide spectrum of plant diseases (Bollen and Fuchs, 1970; Edgington et al., 1971). Application of benomyl or thiabendazole (TBZ) as a soil drench for the control of vascular wilt diseases was found to require extremely large dosages which for many crops are not economical (Erwin, 1973). This was presumably due to tight adsorption of these fungicides to soil and to the immobility of these chemicals in the soil (Baude et al., 1974). The rate of degradation of 2-benzimidazole carbamic acid methyl ester (MBC) and TBZ in the soil was shown to be relatively slow, with a half-life for MBC of several months (Baude et al., 1974).

The mechanism by which these chemicals are bound and/or degraded in the soil is not clear. A study was therefore conducted to examine the mechanism of interaction of benzimidazole derivatives with the soil clay minerals. It has been shown (Mortland, 1970; Bailey and White, 1970) that the acidic environment of clay surfaces provides conditions for protonation of organic bases. Absorption of triazine herbicides or purines and pyrimidines on clay surfaces takes place due to protonation of these molecules (Brown and White, 1969; Weber, 1970b; Yamane and Green, 1972; Lailach and Brindley, 1969).

The effectiveness of soil drenches with benzimidazole fungicides, i.e., the movement and persistence of these chemicals in the soil, may be dependent on their interactions with clay surfaces.

EXPERIMENTAL SECTION

Materials. Chemicals. Analytical standards of TBZ and MBC were obtained from E. Merck and from E. I. DuPont de Nemours & Co. Inc., as were the fungicides recrystallized from technical TBZ and MBC.

Analytical benzimidazole was purchased from BDH Ltd.; magnesium oxide, for chromatography, from BDH Ltd.; aluminum oxide, neutral, activity I, from E. Merck.

Clays. Montmorillonite No. 25 API, Project No. 49 (bentonite), was from Upton Wyoming, and a commercial supply of bentonite was obtained from Upton Wyoming. During this study no real differences were found between the two sources of bentonite and montmorillonite. Ca- and Na-montmorillonite were prepared from Wyoming bentonite according to the procedure described by Shainberg et al. (1975). Kaolinite, Peerless clay No. 2, was from South Carolina.

Adsorption Measurements. The following stock solutions were prepared: TBZ, 10 ppm, and MBC, 5 ppm, dissolved in redistilled water; 0.1 M $CaCl_2$ and 0.1 M NaCl in redistilled water.

The fungicide solution was placed in a 500-ml erlenmeyer flask, $CaCl_2$ or NaCl was added, and the solution was diluted with water to give a final volume of 150 ml

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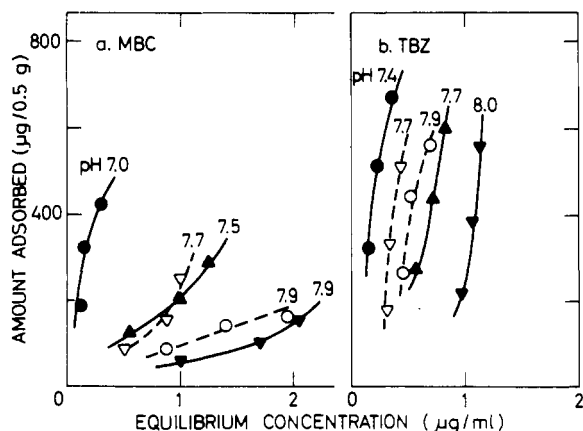


Figure 1. Adsorption isotherms for TBZ and MBC on Ca-bentonite clay at different pH levels. The broken lines connect the points of the corresponding shapes after reversing the pH.

containing various concentrations of the fungicides (0.5–4 ppm of MBC and 0.5–8 ppm of TBZ) in either 0.01 M CaCl₂ or NaCl. To these solutions known amounts of clay were added. Most experiments were run with 0.5 g of clay. The flasks were shaken at ambient temperature (20 ± 1°) for 6 hr. For completion of equilibrium, a period of 2 hr or even less was sufficient. A portion of the equilibrated suspension was centrifuged for 20 min at 10,000 rpm and duplicate samples of 20 ml of the supernatant were taken for analysis.

Adsorption as a function of pH was determined by stirring a clay fungicide suspension of 450 ml with an ionic strength of 0.01 M CaCl₂, the pH being changed by the addition of either HCl or NaOH. The suspension was progressively sampled, after 3 hr of equilibration, duplicate suspension samples of 10 ml each were withdrawn and centrifuged, and the supernatant was taken for analysis. The pH was changed every 30 min by 0.5 pH unit. At the end of each 30-min period, duplicate samples were withdrawn. The solution reached equilibrium within these 30-min intervals; it has been shown that 3 hr of continuous equilibration gave the same result. Variations in concentration between duplicate suspension samples were less than 5%; each experiment was repeated three times.

The quantities of fungicide and clay used in these experiments were: 1500 µg of TBZ and 40 mg of Ca-bentonite; 1500 µg of TBZ and 1.5 g of Na-kaolinite; 1500 µg of MBC and 1 g of Ca-bentonite; and 4500 µg of benzimidazole and 1.5 g of Ca-bentonite.

Analytical Methods. To the clear supernatant (20 ml), 10 ml of 3 N NaOH was added. (If a 10-ml sample was taken, 10 ml of water was added.) The basic aqueous solution was extracted three times with 30 ml of ethyl acetate. Thiabendazole was determined directly from the ethyl acetate phase in a fluorescent spectrophotometer. The excitation wavelength was set at 305 nm and the emission at 345 nm. For the determination of MBC the ethyl acetate phase was evaporated to dryness on a rotary evaporator and the residue was dissolved in 10 ml of absolute ethyl alcohol and measured at an excitation wavelength of 282 nm and emission wavelength at 307 nm. Since benzimidazole is much more soluble in water than TBZ or MBC, it was measured directly in the water by spectrophotometry, at 277 nm for the nonprotonated molecule or at 273 nm for the charged species, at the lower pH.

Extraction of the adsorbed fungicide from the clay was as follows. One gram of clay was shaken for 30 min in 150 ml of aqueous 1 N NaOH. The aqueous phase was extracted three times with 200 ml of ethyl acetate, and the ethyl acetate phase was concentrated to 300 ml and cleaned on an alumina-Celite-magnesium oxide chromatographic column and determined in a fluorescent spec-

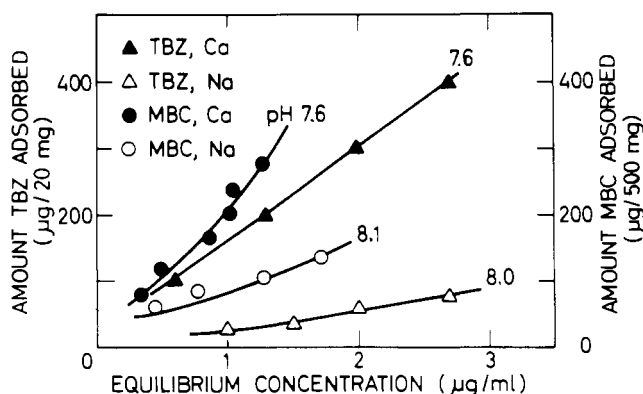


Figure 2. Adsorption isotherms for TBZ and MBC on Ca- and Na-montmorillonite. TBZ adsorbed on 20 mg of clay and MBC on 500 mg of clay.

trophotometer as described previously (Aharonson and Ben-Aziz, 1973).

Recovery of the added fungicides by this analytical procedure was better than 90%. The limit of detection for 1 g of clay was 1–2 µg of MBC and 0.1–0.2 µg of TBZ.

RESULTS AND DISCUSSION

Adsorption isotherms for MBC on Ca-bentonite clay at three different pH levels are presented in Figure 1a. An increase in the acidity of the solution resulted in a significant increase in the adsorption of the fungicide to the clay.

Adsorption isotherms for TBZ (Figure 1b) showed the same pattern of pH dependence, but TBZ was adsorbed in much larger quantities per unit of adsorbent. The adsorption process (solid line, Figure 1) could be reversed by changing the pH of the solution. When the pH of the suspension with MBC was changed from 7.0 to 7.9 or from 7.9 to 7.7, the adsorption isotherms were changed accordingly (broken lines, Figure 1); similar results were obtained for TBZ. It should be stressed, however, that the reversibility of adsorption due to a change of the pH was not complete. The amount of fungicide released from the clay surface was somewhat less than would be expected at each pH. One possible explanation, which is still under study, is that some quantity of the fungicide may be trapped between two or more plates of the bentonite clay.

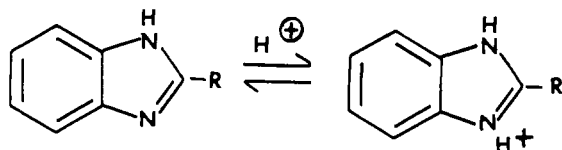
The adsorbed TBZ or MBC was almost completely removed from the clay by shaking the clay with a mixture of 1 N NaOH and ethyl acetate. An average of three extractions showed that 824 µg of TBZ was released from 0.5 g of bentonite on which 873 µg had been adsorbed. The recovery of TBZ was 94%, and the recovery of MBC from the clay was somewhat lower—85–90%.

The effect of the adsorbed cation on the adsorption of TBZ and MBC was studied on Wyoming Na- and Ca-montmorillonite suspensions containing 0.01 M of the respective salt (Figure 2). The larger quantity adsorbed on the Ca clay was probably due to the lower pH of the suspension. In a separate experiment, in which the pH of the Na clay was changed from 8 to 6, there was a sharp increase in the amounts of fungicides adsorbed on the Na clay. Attention should be given to the amounts of adsorbent used for TBZ and MBC, 20 mg for TBZ and 500 mg for MBC, the ratio of which represents the relative strength of adsorption of these two molecules.

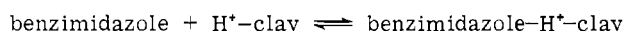
Thiabendazole and MBC were not adsorbed on kaolinite clay under the same conditions as those described previously for Na- and Ca-montmorillonite. The pH of the kaolinite suspension was 7.8. Even at pH 2 only trace amounts of MBC were adsorbed on the clay. On the other hand, an increase in the acidity of the kaolinite suspension resulted in an increase in the adsorption of TBZ. The

amount adsorbed as a function of the pH is presented in Figure 3.

The pH dependent adsorption of benzimidazole derivatives may suggest a reaction with clay surfaces by protonated molecules. The basicity character of these organic molecules is presented in the following formula.



In the presence of increased hydrogen ion activity on the clay surfaces, benzimidazole derivatives may be protonated to form a positive charged molecule which reacts with the clay surface as follows:



Weber (1970a) has shown for triazine herbicides that maximum adsorption of these protonated basic molecules occurs in the vicinity of the ionization constant. The decline in the adsorption at pH lower than the pK_A was explained by the increase in the concentration of hydrogen ions competing for the cation exchange sites. The adsorption curves for benzimidazole derivatives (Figures 3 and 4) did not show a definite maximum adsorption near the pK_A and a decrease at a lower pH.

The shape of the adsorption curves resembles simple titration curves. Almost all the adsorption is confined to four pH units which are needed to convert the nonionized molecules to fully ionized species. The fact that the point of 50% adsorption for TBZ and MBC on Ca-bentonite (Figures 3 and 4) does not correspond to their pK_A values is probably due to the surface acidity of bentonite, which may deviate as much as 3 or 4 pH units lower from the measured pH in the suspension (Bailey and White, 1968). When TBZ was adsorbed on kaolinite (Figure 3), the midpoint of the adsorption curve was very close to its pK_A . This can be explained by the fact that for kaolinite the difference between the surface acidity and the pH of the suspension is much smaller than that of montmorillonite due to its smaller double layer (Van Olphen, 1963). The 50% adsorption for TBZ was found 1.5 pH units higher than that of MBC, whereas the pK_A difference is only 0.5 pH unit. The adsorption curve for TBZ might be due to two pK_A values, one for the benzimidazole ring and a second at a lower pH for the thiazole ring. The pK_A values for benzimidazole derivatives are summarized in Table I.

The pK_A of benzimidazole is 5.5 and that of MBC is 4.0; however, adsorption on bentonite could not be related to the pK_A (Figure 4). Benzimidazole was almost not adsorbed on bentonite when the pH of the suspension was 7, whereas at the same pH MBC was already partially adsorbed on the clay and TBZ was almost completely adsorbed.

It should be pointed out that the solubility of TBZ and MBC in water is very low and quite similar, whereas the solubility of benzimidazole is much higher (at least by a factor of 200). Thus, solubility or hydration of the molecule may be an additional important factor affecting the extent of its adsorption at different pH values.

The main adsorption mechanism seems to be of a cationic nature. The results (Table II) demonstrate for TBZ and for MBC that an increase in the salt concentration (keeping the pH of the solution constant) caused a decrease in the amount of fungicide adsorbed. However, the affinity of the relatively big TBZ ion to the clay surface is greater than that of Ca^{2+} , since considerable amounts are adsorbed in the presence of 1 M CaCl_2 ; 50% of the total TBZ adsorbed on 150 mg of clay. It is possible that forces

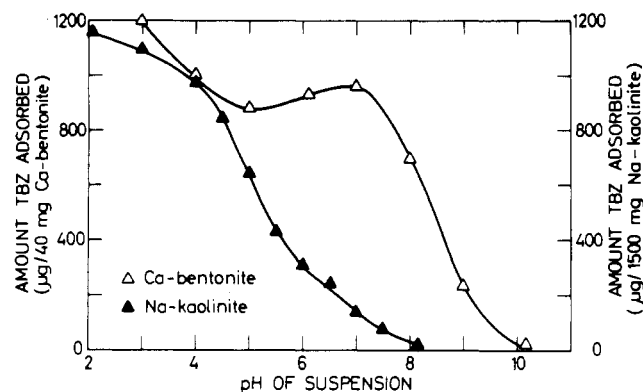


Figure 3. Adsorption of TBZ on Ca-bentonite and Na-kaolinite as a function of the pH of the suspension (ionic strength of the suspension 0.01 M of the respective salt).

Table I. Properties and Structure of Benzimidazole Derivatives Discussed in This Study

Common name	Chemical structure	pK_A^a	Water solubility ^a at pH 7.0
Benzimidazole		5.5 (5.4) ^b	Sparingly
Thiabendazole		(4.7)	Less than 50 ppm
MBC		4.0 (4.2)	Less than 20 ppm
Imidazole		6.9	Freely soluble
Thiazole		2.5	Slightly soluble

^a The pK_A values and water solubility data are from the Handbook of Chemistry, Merck Index, and from E. I. DuPont Inc. ^b Values in parentheses were determined by spectrophotometric titrations.

Table II. Effect of Different Concentrations of CaCl_2 on the Adsorption of TBZ and MBC by Ca-Bentonite

Ca-bentonite, mg/150 ml added,	Amount of fungicide adsorbed, ^a µg	pH of the suspension	CaCl_2 concn, M
MBC			
500	438	269	7.8
500	438	297	7.2
500	438	393	6.8
500	1240	816	6.5
500	1240	441	6.5
500	1240	423	6.5
TBZ			
20	1500	591	5.0
20	1500	420	5.0
20	1500	348	5.0
20	1500	861	7.8
20	1500	285	7.8
20	1500	56	7.8
60	1500	973	7.8
60	1500	460	7.8
60	1500	96	7.8
150	1500	1239	7.8
150	1500	803	7.8
150	1500	735	7.8

^a Results are the average of three measurements.

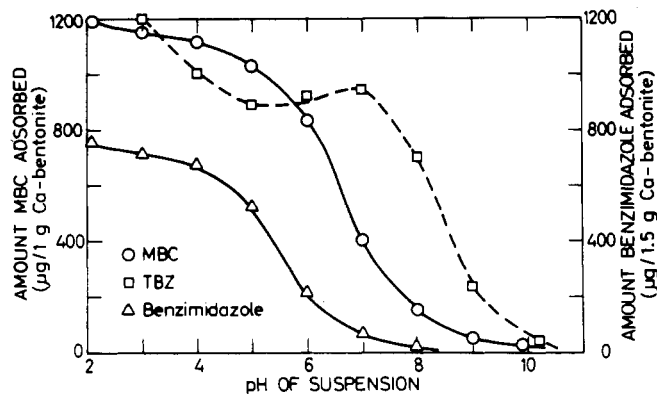


Figure 4. Adsorption of benzimidazole and MBC on Ca-bentonite as a function of the pH of the suspension (ionic strength of the suspension 0.01 M CaCl_2); details for TBZ are as in Figure 3.

Table III. Adsorption of TBZ and MBC on Al_2O_3 and MgO in Aqueous Suspensions

Fungicide	Adsorbent, mg/150 ml	Amount of fungicide added, µg	Amount of fungicide adsorbed, µg	pH of suspension ^a
TBZ	Al_2O_3 , 100	247	0	3.0
TBZ	Al_2O_3 , 100	247	0	8.2
TBZ	Al_2O_3 , 100	247	0	10.8
MBC	Al_2O_3 , 100	220	0	3.0
MBC	Al_2O_3 , 100	220	0	8.0
TBZ	MgO, 200	245	242	10.6
TBZ	MgO, 200	245	221	11.1
TBZ	MgO, 200	245	162	12.2
MBC	MgO, 100	156	136	10.6
MBC	MgO, 100	156	123	11.2

^a The pH of the suspension was changed by the addition of either HCl or NaOH.

other than coulombic ones are operating in the adsorption of the benzimidazole derivatives, and to understand the nature of these forces, further studies are required.

Adsorption of TBZ and MBC on MgO and the effect of pH on this process are shown in Table III. The pH was changed by using either NaOH or HCl. The two fungicides were not adsorbed on alumina oxide. The adsorption of TBZ and MBC on MgO may suggest another specific site of adsorption on montmorillonite clays. It has been shown (Barshad, 1960) that decreasing the pH of a montmorillonite suspension increases the amount of adsorbed magnesium on the clay surfaces.

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Dinitramine: Residues in and Toxicity to Freshwater Fish

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Dinitramine, a herbicide registered for use on cotton and soybeans, is toxic to fish and accumulates in fish tissue after brief exposures to low concentrations. The 96-hr LC_{50} values for several species of coldwater and warmwater fish ranged from 0.590 to 1.52 mg/l. at 12°. Muscle, plasma, and gallbladder bile of carp (*Cyprinus carpio*)

and channel catfish (*Ictalurus punctatus*) were examined for uptake and retention of dinitramine after a 12-hr exposure to a 1 mg/l. concentration. Accumulation in tissues analyzed exceeded the exposure concentration in all instances, and the herbicide was not completely eliminated after the fish had been held in fresh water for 24 hr.

Dinitramine (N^3, N^3 -diethyl-2,4-dinitro-6-trifluoromethyl-*m*-phenylenediamine) is a preplant, incorporated herbicide (U.S. Borax and Chemical Corporation) registered for

use on cotton and soybeans for control of many annual grasses and broadleaf weeds (Berg, 1974). Other names include cobex, USB-3584, and Cobeko.

Newsom and Mitchell (1972) reported on the assay of dinitramine residues in soil and plant tissue. Widespread use of a terrestrial herbicide may lead to the introduction of the compound into surface waters because of runoff, direct application over water, or possible misuse. Therefore, we determined the uptake and retention of dinitramine in carp (*Cyprinus carpio*) and channel catfish (*Ictalurus punctatus*) and the toxicity to several species of fish.

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