Hydrolysis and Adsorption of Cyhalofop-Butyl and Cyhalofop-Acid on Soil Colloids

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A study was undertaken to investigate the stability of cyhalofop-butyl (2*R*)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]butylpropanoate (CyB), an aryloxyphenoxy-propionic herbicide, at different pH values. The hydrolysis of CyB was faster in nonsterile than in sterile water. In sterile medium, CyB degraded only to (2*R*)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propanoic acid (CyA), whereas in nonsterile water, also the metabolites (2*R*)-2-[4-(4-carbamoyl-2-fluorophenoxy)phenoxy]propanoic acid (CyAA) and (2*R*)-2-[4-(4-carboxyl-2-fluorophenoxy)phenoxy]propanoic acid (CyD) were detected. The adsorption of CyB onto clays, iron oxide, and dissolved organic matter (DOM), using a batch equilibrium method, was also studied. A lipophilic bond is responsible for CyB adsorption on DOM. CyB was adsorbed on Fe^{III}- and Ca-clays through hydrogen bonding between the carbonyl oxygen and water surrounding the exchangeable cations. In the interlayer of K-clay, CyB was hydrolyzed to CyA, which remained adsorbed therein as a monomer. The acid CyA was adsorbed only by the Fe-oxide through complexation. The CyA−Fe-oxide complex was stable and did not undergo degradation.

KEYWORDS: Cyhalofop-butyl; cyhalofop-acid; hydrolysis; adsorption; clays; iron oxide; DOM

INTRODUCTION

Cyhalofop-butyl (CyB, **Figure 1**) is an aryloxyphenoxy-propionate (AOPP) herbicide for the postemergence control of barnyard grasses in rice paddies (*1, 2*). At application rates of 210 g ha^{-1} of active ingredient (*3*), CyB controls almost all *Echinochloa* species. Similar to other AOPP herbicides, the mode of action is related to the inhibition of acetyl coenzyme A carboxylase (ACCase, EC 6.4.1.2), a key enzyme involved in the fatty acid biosynthesis (*4*). The herbicide is formulated as an ester to facilitate the movement through the plant cuticle, but once in the plant, it rapidly hydrolyzes to cyhalofop-acid (CyA, **Figure 1**). CyA is the primary metabolite of CyB and also the herbicidally active form (*5*). AOPP herbicides undergo a pH-dependent hydrolysis of the ester linkage to give the parental acids, which generally are weak acids with pK_a values ranging from 3 to 5 (*6*). In aqueous solution, these herbicides exist primarily in the neutral form at pH values below pK_a and in the anionic form at pH above pK_a. Therefore, in most agricultural soils, they are predominantly anionic. Degradation and retention processes are among the most important factors influencing the fate of an agrochemical in soil environment. Degradation is the result of both microbial and chemical processes. Hydrolytic reactions are common in surface waters

(*7*); moreover, pesticide hydrolysis is often promoted by adsorption on soil colloids (*8*).

To our knowledge, no information is available about the CyB adsorption on soil colloids and its hydrolytic behavior. This work describes the kinetics of CyB hydrolysis in sterile and nonsterile aqueous buffers over the pH range of 4−9 and the nature of degradation products. The adsorption of CyB onto homoionic clays, iron oxide, and dissolved organic matter (DOM) was also studied. The adsorption study was extended to the acid CyA, which is the primary metabolite of CyB. On the basis of the spectroscopic results, interaction mechanisms are proposed.

MATERIALS AND METHODS

Materials. Cyhalofop-butyl (99.9% purity) was supplied by Dow Agrosciences B. V. Rotterdam (The Netherland). CyB purity was checked by high-performance liquid chromatography (HPLC). CyB water solubility is 0.44 mg L^{-1} at pH 7 (*9*).

The metabolites cyhalofop-acid (CyA, 99.4%), cyhalofop-amide (CyAA, 99%), and cyhalofop-diacid (CyD, 98%) (**Figure 1**) were kindly supplied as analytical standards by Dow Agrosciences, Bologna, Italy.

The amounts of cyhalofop-acid needed for the adsorption study were prepared by alkaline hydrolysis of CyB according the following procedure: a NaOH solution (0.5 N) was added to 100 mL of suspension of CyB (1 g) in CH$_3$CN + H$_2$O (50 + 50, v/v) up to pH 9 under stirring. The suspension was kept at room temperature until the ester was completely dissolved at pH 9. Then, CH$_3$CN was evaporated, and HCl (0.1 N) was added to the crude reaction mixture up to pH 2. The acid, precipitated as a white solid, was filtered and recrystallized from hydro-alcoholic solution. Sodium (2*R*)-2-[4-(4-cyano-2-fluorophenoxy)phe-

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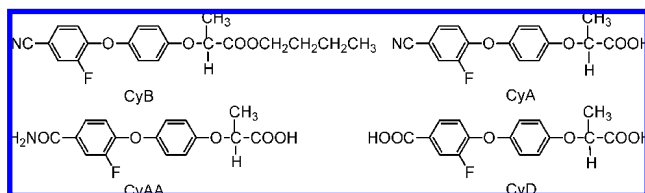**5274** *J. Agric. Food Chem.,* Vol. 56, No. 13, 2008

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Figure 1. Chemical structure of CyB and its metabolites.

noxy]propanoate (CyNa) was prepared by adding a water solution of CyA (31 mg) to a 2 mL sample of 0.05 M NaOH at room temperature. After 3 h, water was evaporated under vacuum and white crystals of CyNa were obtained. IR (KBr) ν (cm^{-1}): 2984, 2937, 2237, 1600, 1501, 1419, 1287, and 1217.

Montmorillonite number 26 (bentonite) from Crook County, WY, supplied by Ward's Natural Science Establishment, Rochester, NY, was used. The <2 μm fraction was obtained by sedimentation. The cation-exchange capacity (CEC) of the sodium form determined by the method of Hendershot and Duquette (*10*) was 0.902 eq kg^{-1}. Fe^{3+}-, Ca^{2+}-, and K$^+$-exchanged samples were prepared by repeated treatments of the clay with 1 N solutions of respective metal chlorides. The samples were centrifuged, washed with deionized water until Cl$^-$ free, and dried at room temperature.

Hydrated ferric oxide was prepared by adding 0.50 mol of FeCl$_2$ to an equivalent amount of 2 M KOH (250 mL) under rapid stirring. The precipitate was washed with distilled water and dried under vacuum. It was amorphous according to X-ray analysis and completely soluble in ammonium oxalate (pH 3).

The sediment sample was collected from a paddy field near Novara (Italy). DOM was extracted by shaking the sediment (1 kg, <2 mm fraction) overnight with 2 L of distilled water. Then, the suspension was filtered through a 0.45 μm Durapore membrane filter (Millipore), and the filtrate was freeze-dried. In general, 1 kg sample of sediment gave about 200 mg of DOM.

CyB working solutions were prepared by dissolving ca.10 mg of CyB in 10 mL of acetonitrile.

Buffer aqueous solutions (Merck), prepared with sterile and nonsterile deionized water, at pH 4, 7, and 9 were used.

All of the solvents were of HPLC grade (Carlo Erba Reagenti, Milano, Italy) and were used without further purification.

CyB and CyA Hydrolysis. The hydrolysis rate was determined by monitoring the disappearance of CyB and CyA in buffered aqueous solutions. CyB hydrolysis was studied both in buffered sterile (0.53 mg L^{-1}) and nonsterile (0.57 mg L^{-1}) solutions, whereas CyA hydrolysis was carried out only in nonsterile solution (4.27 mg L^{-1}). CyB sterile solutions were prepared by autoclaving the buffer solutions at 121 °C and 2 atm for 30 min and then adding appropriate amounts of working CyB solution under a sterile hood. The solutions were maintained in the dark at 25 °C. At appropriate times, each test solution was analyzed directly by HPLC. All of the experiments were run in triplicate.

CyB and CyA Sorption. CyB and CyA sorption was performed on different colloidal systems (Fe^{3+}-, Ca^{2+}-, and K$^+$-clays, Fe-oxide, and DOM) using a batch equilibrium method. In general, samples of colloid (25 mg) were equilibrated in polyallomer centrifuge tubes with 10 mL of aqueous CyB (0.25–0.53 mg L^{-1}) or CyA (1.50–3.13 mg L^{-1}) solutions. The tubes were shaken in an end-over-end shaker (70 rpm) at 25 ± 2 °C. After equilibration, the suspensions were centrifuged at 19000g for 15 min and the supernatant was pipetted off and analyzed immediately by HPLC. The equilibrium time was determined through a kinetic study using 0.53 and 3.10 mg L^{-1} CyB and CyA solutions, respectively. The amount adsorbed by the colloid was calculated from the difference between the initial and final concentrations of CyB or CyA in solution. The tubes were previously checked to exclude the adsorption of either CyB or CyA. All of the experiments were run in triplicate.

CyA Degradation on Colloids. CyA degradation was studied on colloidal aqueous suspensions (Fe^{3+}-, Ca^{2+}-, and K$^+$-exchanged clays, Fe-oxide, and DOM). In general, 25 mg samples were suspended in CyA aqueous solution (10 mL, 3.10 mg L^{-1}) in polyallomer centrifuge

Table 1. Kinetic Parameters for CyB Degradation in Nonsterile and Sterile Aqueous Conditions at Different pH Values

pH	k_{obs} (day^{-1})	$t_{1/2}$ (day)	r
	Nonsterile Conditions		
4.0	1.4×10^{-1}	4.8	0.9924
7.0	6.9×10^{-1}	1.0	0.9995
9.0	8.7×10^{-1}	0.8	0.9916
	Sterile Conditions		
4.0	2.0×10^{-3}	347	0.9926
7.0	8.2×10^{-3}	85	0.9954
9.0	3.8×10^{-1}	2	0.9920

tubes. The samples were kept at 25 ± 2 °C under stirring. Kinetic studies were carried out by removing 0.5 mL aliquots at different times. The suspensions were ultracentrifuged, and the degradation products were measured by HPLC. The experiments were run in duplicate.

HPLC Analyses. The concentration of CyB and its degradation products was determined by HPLC. The system was assembled as follows: a Waters 1515 pump equipped with a Waters 2487 UV/vis programmable detector operating at 250 nm, a Breeze chromatography software, and a μBondapak C$_{18}$ analytical column (10 μm, 3.9 × 300 mm). The mobile phase was acetonitrile plus water (70 + 30 by volume, pH 2.7) at a flow rate of 1 mL min^{-1}. The retention times under these chromatographic conditions were 2.9, 3.2, 3.9, and 9.7 min for CyAA, CyD, CyA, and CyB, respectively. The quantitative determination of CyB and its metabolites, measured as an average value of triplicate injections, was performed by using external standards ($r_{\text{working curve}}$ = 0.9989). Calculations were based on the average peak areas of the external standards. The detection limit for CyB was 0.1 mg L^{-1}, namely, the herbicide concentration corresponding to a detector response approximately twice the background signal.

Infrared Analyses. Fourier transform infrared (FTIR) spectra were recorded with a Nicolet 205 spectrophotometer over the range of 4000−600 cm^{-1}. The spectra of CyB, CyA, CyNa, CyA−Fe^{III}, and CyA−Fe-oxide complexes were recorded on KBr disks. The spectra of Fe-, Ca-, and K-clay−CyB and K-clay−CyA complexes were recorded on self-supporting films. These films were prepared by evaporating 5 mL of an aqueous clay suspension (2 g of clay in 100 mL of H$_2$O) on a polyethylene sheet at room temperature, then air-dried, and divided into two parts. One piece was placed into a CyB or CyA CHCl$_3$ solution (2%) for 24 h, then removed, rinsed several times with pure solvent, and air-dried. The other one was treated similarly but only with pure CHCl$_3$. The IR spectra of the clay complexes were recorded immediately after air drying.

Differential spectra were obtained by subtracting the spectrum of the proper blank clay film from those of the treated clay complex. Only IR absorptions in the range of 2000−1200 cm^{-1} are discussed for clay complexes because this region provides evidence for the sorption mechanisms. All of the spectra were the means of at least 64 scans at 4 cm^{-1} resolution, which gives a reasonably good signal-to-noise ratio.

Data Analysis. Kinetic data were fit to a first-order rate law, ln(C/C_0) = $-k_{obs}t$, where C is the herbicide concentration (in micromolar units) at the t time, C_0 is the initial herbicide concentration, and k_{obs} is the first-order rate degradation constant.

RESULTS AND DISCUSSION

CyB Hydrolysis. The hydrolysis of CyB was studied at pH values of 4, 7, and 9 in nonsterile and sterile solutions (**Table 1**). The degradation was faster in nonsterile than in sterile medium. Analogous results were observed in the soil by Jackson and Douglas (*9*), which found that CyB is completely degraded within 1 h in nonsterile soil, whereas it was stable in sterile conditions. Both in nonsterile and sterile conditions, the increase of pH favored CyB degradation, but only at a pH value of 9, the degradation rates were comparable. Most likely, this pH is not suitable for biotic CyB transformation; therefore, only the abiotic degradation occurred.
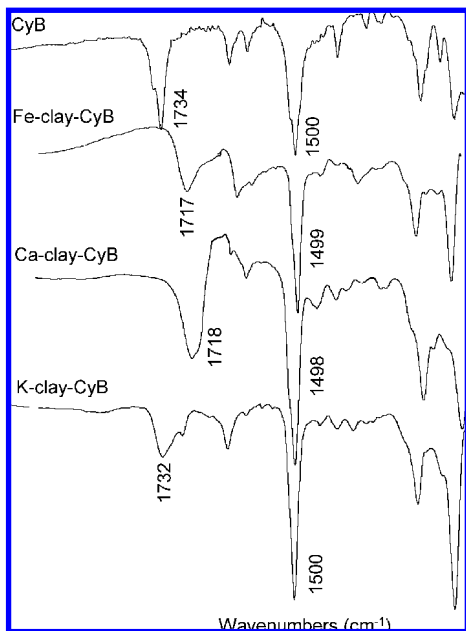
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Figure 2. FTIR spectra of CyB and its clay complexes.

During sterile hydrolysis, CyA was the only metabolite detected, whereas in nonsterile medium, also CyAA and CyD were observed. This suggests that CyAA and CyD byproducts arise from biological activity.

CyB Sorption on Colloids. CyB adsorption was rapid on all colloids tested. After a few minutes, CyB was totally depleted from suspensions. Therefore, we could not measure the adsorption isotherms. The scarce solubility of herbicide in water can explain the adsorption on DOM, most likely because of a lipophilic effect. Evaluating the mechanisms involved in the CyB adsorption on clays from aqueous solutions by FTIR is a difficult task, because at low CyB concentrations, the water competes with herbicide for surfaces. Therefore, the adsorption study was carried out from $CHCl_3$. The FTIR spectra of CyB and Fe^{III}-, Ca-, and K-clay complexes are reported in **Figure 2**.

The spectra indicate that the herbicide undergoes significant changes upon interacting with montmorillonite. CyB exhibits an absorption at 1734 cm^{-1} because of the stretching mode of the carbonyl group. The FTIR spectra of CyB$-Fe^{III}$- and CyB$-$Ca-clay complexes are very similar to each other and show an absorption at 1717$-$1718 cm^{-1} for the carbonyl group. This displacement to lower wavenumbers suggests a decrease in the double-bond character of the C=O group, most likely because of a hydrogen bond to hydration water of the exchangeable cations. The spectrum of the CyB$-$K-clay complex displays bands different from those observed for both CyB$-Fe^{III}$- and CyB$-$Ca-clay complexes but similar to those of neat CyB. The film of the K-clay complex was washed many times with chloroform, but the FTIR spectrum did not change. This finding suggested that a compound with spectral features similar to those of CyB was trapped in the K-clay interlayer. To identify this compound, the FTIR spectra of CyB metabolites adsorbed on K-clay were recorded. Only the spectrum of the CyA$-$K-clay complex (**Figure 3**) was practically similar to that of the CyB$-$K-clay complex (**Figure 2**). This suggests that, upon adsorption on K-clay, CyB is hydrolyzed into parental acid CyA that remains adsorbed in the interlayer. The increase of frequency of the C=O stretch from 1720 cm^{-1} of neat acid to 1731 cm^{-1} in the K-clay suggests that a monomer is present in the clay (**Figure 3**). In fact, monomeric acids exhibit C=O absorptions at higher frequencies than dimeric acids, because the hydrogen
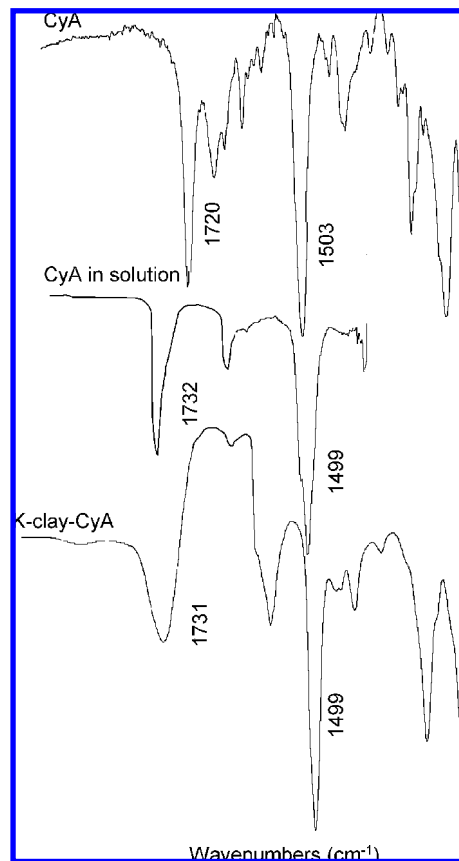

Figure 3. FTIR spectra of CyA in KBr, in CH_2Cl_2, and adsorbed on K-clay.

Table 2. Kinetic Parameters for CyA Degradation in Nonsterile Aqueous Solutions at Different pH Values and in the Presence of Fe-clay and DOM

system	pH	k_{obs} (day^{-1})	$t_{1/2}$ (day)	r
water	4.0	2.3×10^{-3}	301	0.9927
water	7.0	5.6×10^{-2}	12	0.9973
Fe-clay	4.2	2.9×10^{-2}	24	0.9966
DOM	7.3	8.7×10^{-2}	8	0.9946

bond of the dimer weakens the C=O bond (*11*). This effect may be observed by comparing the FTIR spectrum of CyA in methylene chloride with that obtained in KBr (**Figure 3**). The solvent prevents the formation of hydrogen bonding, and the C=O frequency is observed at 1732 cm^{-1} in the organic solution compared to 1720 cm^{-1} in the solid state. Most likely, the acid is present as a monomer in K-clay because the interlayer thickness hinders the accommodation of the bigger dimeric form.

No evidence on the adsorption mechanisms of CyB on iron oxide and DOM could be obtained by FTIR, because the bands of the colloidal substrates masked those as a result of herbicide.

CyA Sorption and Degradation on Colloids. CyA is the primary metabolite of CyB and the biologically active molecule. No significant adsorption of CyA occurred on clays and DOM. CyA is a weak acid with a pK_a value of 3.8 (*12*); therefore, at the pH values of clays and DOM suspensions (**Table 2**), the acid is dissociated. The repulsion between CyA anionic molecules and the negatively charged surfaces explains the lack of CyA adsorption on clays and DOM. Although the Fe-saturated clay and DOM did not adsorb the acid, the CyAA and CyD degradation products were observed with elapsing time. The kinetic parameters at different pH
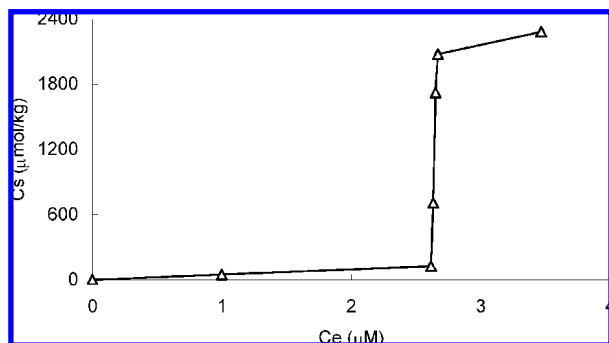
Figure 4. Adsorption isotherm of CyA on iron oxide.

values and in the presence of Fe-clay and DOM, shown in **Table 2**, indicate that, at comparable pH values, Fe-clay is more effective in promoting CyA degradation. Water surrounding Fe-clay surfaces is more acidic than the molecules in bulk solution because of the high polarizing power of iron. This greater surface acidity could therefore be responsible for the higher degradation extent. A separate CyA hydrolysis experiment was carried out to find the pH solution value necessary to observe a CyA $t_{1/2}$ similar to that measured in the presence of Fe-clay (24 days). At a pH value of 2, $t_{1/2}$ for CyA was of 23 days. This finding supports that the suspension close to the clay surfaces is more acidic than the bulk solution.

On the other hand, CyA adsorption was rapid on iron oxide and the equilibrium was attained within 1 h. The zero-point charge of iron oxides is around pH 8.5 (*13*); therefore, in the pH range of the studied systems, only iron oxide exhibits positively charged surfaces. The adsorption of herbicides on iron oxide is generally attributed to complex formation following the replacement of a hydroxyl group bound to the iron atom with an organic functional group (*14*).

Actually, an unusual shape is shown by the adsorption isotherm of CyA on iron oxide (**Figure 4**). At low concentrations, CyA did not show detectable affinity for the oxide. On the contrary, above ≈2.6 μM concentration, the affinity of CyA for the oxide was strong and the acid was largely removed from solution. The FTIR spectrum of the CyA−iron-oxide complex is shown in **Figure 5**. The spectrum did not show bands attributable to an un-ionized carboxyl group. Moreover, bands assignable to the ν_{as}(COO) and ν_{sym}(COO) modes of the carboxylate group appeared at 1616 and 1419 cm^{-1}, respectively. These results suggest complex formation. Therefore, we synthesized the Fe^{III}−CyA complex: as shown in **Figure 5**, the spectra of the CyA−iron oxide and Fe^{III}−CyA complexes are similar. Although the structural study of the CyA−iron oxide complex is beyond the scope of this work, we compared the spectra of the sodium salt and the iron complex of CyA. The difference value between ν_{as}(COO) and ν_{sym}(COO) frequencies of the sodium salt ($\Delta\nu = 181$ cm^{-1}) is close to the corresponding value of the iron complex ($\Delta\nu = 197$ cm^{-1}). This could be evidence for bridged complexes (*15*). The shape of the CyA adsorption curve on iron oxide could be explained in the following way: at low CyA concentrations, the lack of adsorption reflects an incorrect stoichiometry between ligand and iron, and if the CyA concentration increases, the formation of the complex favors the adsorption.

No CyA degradation was detected in the presence of iron oxide. Most likely, the iron complexation prevents the degradation of the acid. In conclusion, despite the incomplete knowledge of processes occurring on the colloids, the results indicate that CyB may be removed and degraded by rich colloid soils. This
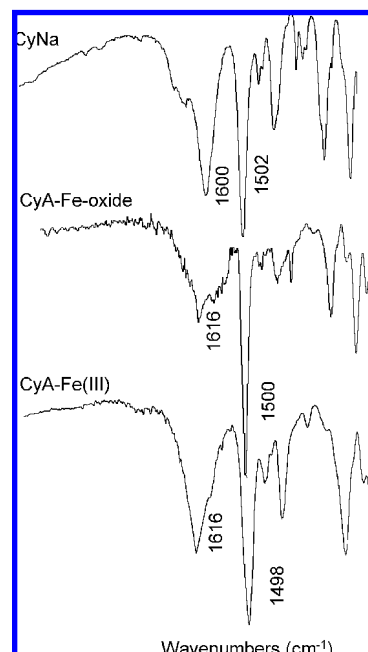

Figure 5. FTIR spectra of CyA sodium salt and CyA−Fe complexes.

may be particularly significant for a pesticide for which scarce information is reported.

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