Depth Dependence of Direct and Indirect Photolysis on Soil Surfaces

Vincent R. Hebert† and Glenn C. Miller*

Department of Biochemistry, University of Nevada, Reno, Nevada 89557

The photolysis depth of direct and indirect photolysis in soils was determined with use of two agrochemicals. The dinitroaniline herbicide flumetralin and a dialkyl thioether organophosphorus insecticide disulfoton were homogeneously applied to four soils and irradiated. Flumetralin undergoes direct photolysis while disulfoton undergoes indirect photolysis by reaction with singlet oxygen. Various depths of treated soils (0.4–4.0 mm) were exposed to natural sunlight and laboratory lighting until no further degradation of chemical was detected. An estimate for the depth of photolysis was determined by multiplying the soil depth by the percent loss of each chemical. Direct photolysis was found to be restricted to the photic depth of soils (0.2–0.4 mm), while the indirect photolysis depth was slightly deeper. Vertical migration of singlet oxygen to depths greater than the depth of light penetration appears likely, although greater chemical movement of the more volatile disulfoton may account, in part, for the enhancement in indirect photolysis. In all cases photolysis was limited to less than 1.0 mm for laboratory irradiations. Irradiation of the soil in sunlight resulted in greater depths of photolysis (up to 2 mm).

The photochemical degradation of pollutants at the atmosphere–soil interface can be important for surface-applied agrochemicals. On dry, sunlight-exposed surfaces, photolysis may dominate other transformation pathways that are favored under conditions found in the bulk soil (Smith et al., 1978). Direct photolysis rates are substantially slower for soil-sorbed pesticides in comparison to rates in distilled water, presumably due to light attenuation by the soil (Nilles and Zabik, 1975; Miller and Zepp, 1983). Although the rates are slower, residence times for many photolabile pesticides at this interface will be significant and photolysis can proceed at significant rates in relation to other fate processes.

Indirect photochemical processes may also affect the fate of surface-applied agrochemicals. A variety of oxidants are formed on the sunlight-exposed soil surface that can potentially transform xenobiotics (Pohlman and Mill, 1983). Studies have shown that singlet oxygen is formed on the irradiated soil in significant yields (Gohre and Miller, 1983). This oxidant will convert many sulfide-containing pesticides to their sulfoxide analogues at environmentally significant rates (Gohre and Miller, 1986). These photoproducts are often more persistent, more toxic, and more water soluble than the parent pesticide.

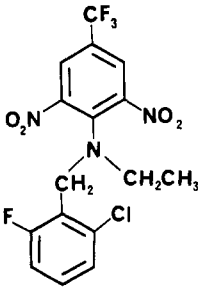
Although successful models have been developed for predicting the fate of pesticides and organic contaminants in transparent media (Zepp and Cline, 1977), assessing the photochemical fate of xenobiotics at the soil–atmosphere interface is more difficult. Competitive sunlight absorption by soil chromophores (Hautala, 1978), variable sorption of pesticides on organic and inorganic soil colloids, and competing biotic and abiotic transformation processes complicate the use of simple models for predicting photolysis rates at the soil surface. In addition, thermal heating at the soil interface enhances the convective and evapotranspirative transport of hydrophobic organics to the surface and can complicate photochemical kinetics.

Previous studies measuring pesticide photolysis rates have been performed with use of thin soil layers of varying organic content (Smith et al., 1978; Takahashi et al., 1985; Mikami et al., 1980). Although these studies address the contributions of various soil constituents in photodecomposition, little progress has been made in developing a reliable method for predicting photolysis rates of low volatility pesticides on sunlight-exposed soil under environmental conditions. The purpose of this present study is to better define the significance of sunlight-induced transformations at the soil surface by evaluating the influence of soil depth on direct and indirect photochemical processes.

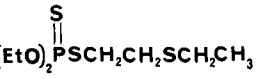
MATERIALS AND METHODS

Soil Preparation. Characterizations of sandy loam soils from four agricultural areas used in this study are presented in Table I. These soils were chosen on the basis of their variabilities in organic matter content. Each soil was air-dried in the laboratory and sieved through a 425-μm sieve screen.

Pesticides. Flumetralin, *N*-ethyl-*N*-(2-chloro-6-fluorobenzyl)-2,6-dinitro-α,α,α-trifluoro-*p*-toluidine (98.8%), was provided by CIBA-GEIGY Corp., and disulfoton, *O*,*O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate (>95%), was obtained from Mobay.



flumetralin



disulfoton

Photolysis Depth Dependence. Each of the soils was coated with a methylene chloride solution of flumetralin and disulfoton. The solvent was removed slowly from the soil slurry by rotary evaporation at 40 °C. This method gave a uniform pesticide distribution in the soil at 100 mg/kg. For each treated

† Present address: FMC Corp., Richmond, CA.

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Table I. Soil Properties

soil[a] designation	% organic matter	pH	% clay	% silt	% sand	bulk density
Kracaws	0.8	7.5	8	47	45	1.28
Main Station Farm	2.0	7.2	14	32	54	1.32
Montana Grain	2.2	7.6	22	28	50	1.60
Chico Orchard	6.1	6.5	14	31	55	1.35

[a] Soils were collected from the following locations: Kracaws, 40 km north of Winnemucca, NV; Main Station Farm, University of Nevada research farm in Reno, NV; Montana Grain, 40 km south of Plentywood, MT; Chico Orchard, walnut orchard 4 km west of Chico, CA.

soil, 4-, 8-, 16-, and 32-g portions were evenly spread on the bottom of glass Petri dishes (10-cm diameter). Uniform depths at each measured amount were estimated from soil bulk density measurements (Table I) and Petri dish area. For each of the four soil depths, 10 dishes were prepared. Five of these dishes were exposed to light, and the other five were used as unexposed (dark) controls. All dishes were covered with 0.5-mil transparent polyethylene film to minimize losses during outdoor exposure. The transparency of the polyethylene film to sunlight wavelengths was established by measuring the absorbance spectrum. Sheet metal trays (35 cm × 25 cm × 3 cm) partially covered with black poster board were used to house each set of four soil depths. These trays were fastened to the ground, which aided in minimizing surface mixing from wind turbulence. Dark control trays were covered with 4-mil black polyethylene film.

Irradiations were conducted in the outdoors in Reno, NV, between April and September (1985 and 1986). One sunlight-exposed and one dark control tray were removed at 24-h intervals and placed in cold storage until the end of the 5-day irradiation period.

For laboratory irradiations, soils and Petri dishes were prepared as previously described. Petri dishes were placed on a 400 cm × 40 cm grid consisting of 40 10 cm × 10 cm quadrants under eight 4-ft fluorescent FS40 Westinghouse sunlamps. The uniformity of the light field in each of 40 quadrants was determined by measuring the photolysis rate of flumetralin (10 mg/L) in acetone at each position. Quadrants that appreciably deviated more than 5% from mean light intensity were not used to irradiate samples. Electric fans were positioned under the light bank to stabilize temperatures between 28 and 31 °C during irradiation. A complete set consisting of four soil depths and their respective aluminum foil covered dark controls were removed at 48-h intervals and placed in cold storage until the end of the 192-h irradiation period. Recovery of dark controls varied from 87 to 104%.

Pesticide Quantitation. Soil samples were transferred from Petri dishes to 100-mL beakers and homogenized. A 1-g subsample from each beaker was extracted three times with 4 mL of acetone and analyzed by using a Hewlett-Packard 5830 gas chromatograph equipped with a nitrogen–phosphorus detector. Resolution of the analytes was achieved on a 2 m × 2 mm (i.d.) borosilicate glass column containing 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport.

RESULTS AND DISCUSSION

Photolysis Depth Dependence. Preliminary studies were conducted measuring light transmission on thin layers of soil. Very thin layers of soil from three of the designated areas were irradiated on borosilicate dishes with xenon arc radiation. A spectroradiometer was used to measure the light passing through the soil. When a comparison was made between light transmission of soils versus Pyrex, these studies indicated light is dramatically attenuated at very shallow depths. Ultraviolet light in all irradiated soils was found to be greater than 90% attenuated in the top 0.2 mm. Figure 1 presents the results of one of these irradiations conducted on the Chico soil. Although this technique was semiquantitative, it provided an upper limit of light penetration into the soil.

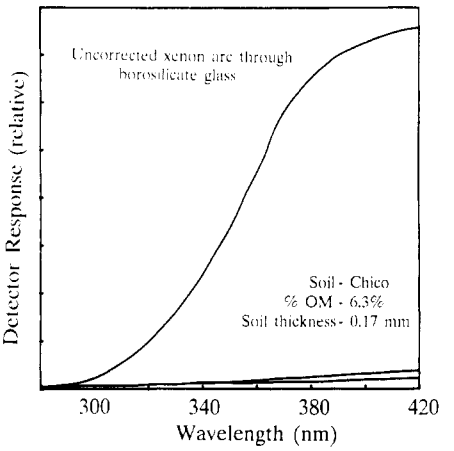

Figure 1. Transmission of xenon arc light through 0.17-mm-thick Chico soil. The lines at the base of the graph represent duplicate measurements.

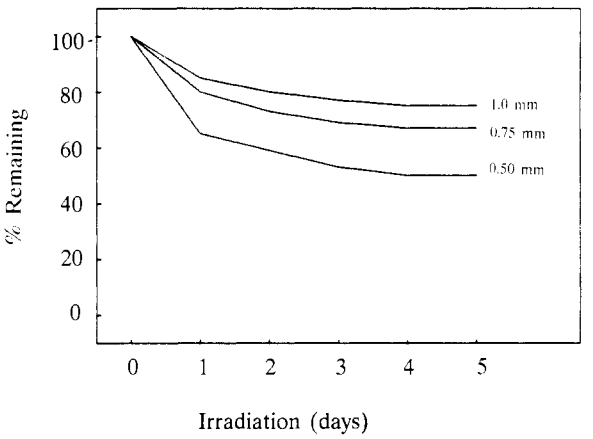

Figure 2. Idealized model for photolysis of pesticides on different thicknesses of a soil where the mean photolysis depth is 0.25 mm.

Direct photolysis is expected to be similarly restricted to the same vertical region.

Figure 2 illustrates an idealized depiction of mean photolysis depth at various exposed soil thicknesses under the following conditions: (1) Light penetration is spectrally homogeneous to the limit of its optical depth and behaves in a Beer–Lambert fashion. (2) Pesticide distribution is initially homogeneous throughout the soil material. (3) Pesticide movement is insignificant. (4) Direct photolysis is the only transformation process. The mean depth of photolysis is calculated as the percentage loss of the compound multiplied by the soil depth. This method assumes that the photochemically available compound is lost, leaving the fraction protected from photolysis. The percent of pesticide transformed by direct photolysis at each depth should independently estimate actual photolysis depth.

The dinitroaniline herbicide flumetralin was selected to estimate the mean depth dependence of direct photolysis. The photochemical properties of flumetralin are similar to those of trifluralin (Leitis and Crosby, 1974). The vapor pressure of flumetralin, however, is substantially lower (less than 1×10^{-6} mmHg at 20 °C, CIBA-GEIGY). Solution photolysis studies conducted in natural sunlight have shown that flumetralin undergoes rapid direct photolysis with a midday midsummer half-life of 20 min (Miller unpublished).

The results from exposing soils of various depths containing flumetralin under sunlight and FS40 lamps are presented in Figures 3–6 and Tables II–V. On all irra-

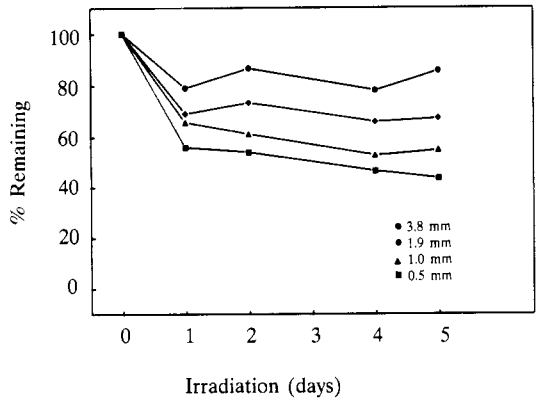

Figure 3. Sunlight photolysis of flumetralin on various thicknesses of Kracaws soil.

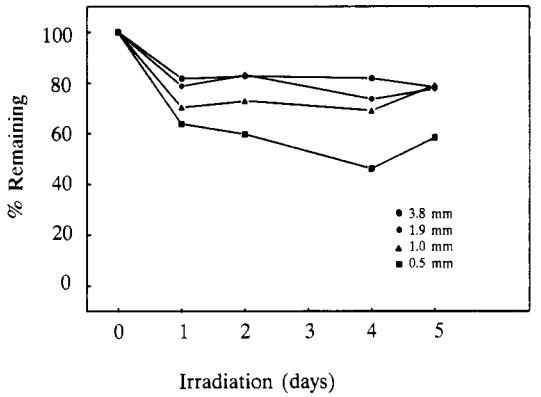

Figure 4. Sunlight photolysis of flumetralin on various thicknesses of Main Station Farm soil.

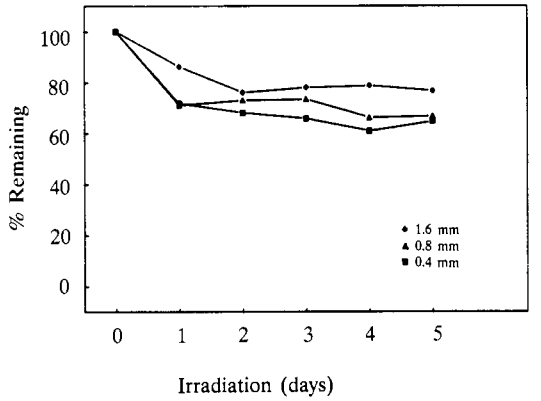

Figure 5. Sunlight photolysis of flumetralin on various thicknesses of Montana soil.

diated soils, the loss of flumetralin was significantly slower than solution photolysis rates. In most instances, greater than 50% of the original concentration was recovered after 5 days of sunlight irradiation. A rapid initial loss of flumetralin was observed, but generally from day 3 to the termination of the experiment, no further significant loss was evident. Presumably, after the third day all of the chemical available to direct photolysis was transformed.

Direct sunlight photolysis of flumetralin in the soil did not follow simple first-order kinetics. This is a general characteristic of low-volatility pesticides in soils (Gohre and Miller, 1986). The relative loss among soil depths, however, provides a basis for evaluating the depth dependence of photolysis. For each soil in Figures 3–6, a pattern was apparent among irradiated soil depths analogous to the idealized model presented in Figure 2. Although a depth-dependent relationship was clearly

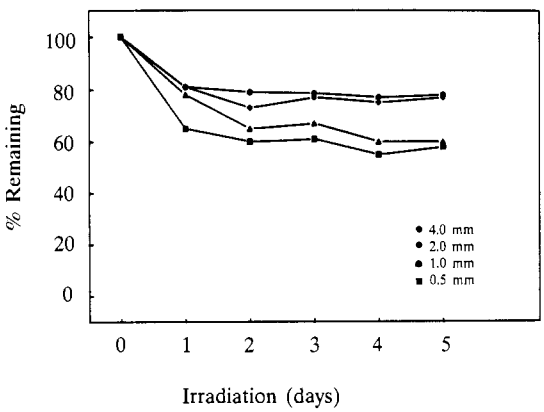

Figure 6. Sunlight photolysis of flumetralin on various thicknesses of Chico orchard soil.

Table II. Estimated Mean Photolysis Depths in Kracaws Soil

soil depth, mm	estimated photolysis depth, mm	
	outdoor	indoor
A. Disulfoton (Indirect Photolysis)		
0.5	0.2	0.2
0.9	0.3	0.2
1.9	0.5	0.2
3.8	0.6	0.4
B. Flumetralin (Direct Photolysis)		
0.5	0.2	0.1
0.9	0.3	0.2
1.9	0.6	0.2
3.8	0.8	0.2

[a] Estimates of photolysis depth were derived by multiplying the percent recovery of starting material by soil depth when photolysis rates approached zero.

Table III. Estimated Mean Photolysis Depths in Main Station Farm Soil

soil depth, mm	estimated photolysis depth,[a] mm	
	outdoor	indoor
A. Disulfoton (Indirect Photolysis)		
0.5	0.2	0.1
1.0	0.4	0.3
1.9	0.7	0.2
3.8	0.7	0.4
B. Flumetralin (Direct Photolysis)		
0.5	0.1	0.1
1.0	0.1	0.2
1.9	0.2	0.2
3.8	0.4	0.2

[a] Estimates of photolysis depth were derived by multiplying the percent recovery of starting material by soil depth when photolysis rates approached zero.

apparent, conformity to ideal depth behavior was not observed.

Estimated photolysis depths for the various soil thicknesses varied with the depth of soil. At depths greater than 1 mm, direct photolysis depth estimates among sunlight irradiated soils were generally found to be greater than depth estimates for depths less than 1 mm (Tables II–V). Temperatures measured at the surface of sunlight-exposed soils typically exceeded 40 °C at noon. At these temperatures, pesticide movement at depths below the soil optical zone may have accounted for greater photochemical availability at the exposed surface of the deeper soils. Although the soils used in this study were air-dried, convective transport to the soil–atmosphere interface has been previously reported to be a significant process. Even at the most shallow soil depths, not all of the

Table IV. Estimated Mean Photolysis Depths in Montana Grain Soil

soil depth, mm	estimated photolysis depth,[a] mm outdoor	indoor
	A. Disulfoton (Indirect Photolysis)	
0.4	0.3	0.2
0.8	0.4	0.3
1.6	0.6	0.2
3.1	0.8	0.4
	B. Flumetralin (Direct Photolysis)	
0.4	0.1	0.1
0.8	0.1	0.1
1.6	0.2	0.2
3.1	0.1	0.2

[a] Estimates of photolysis depth were derived by multiplying the percent recovery of starting material by soil depth when photolysis rates approached zero.

Table V. Estimated Mean Photolysis Depths in Chico Orchard Soil

soil depth, mm	estimated photolysis depth,[a] mm outdoor	indoor
	A. Disulfoton (Indirect Photolysis)	
0.5	0.3	0.2
1.0	0.6	0.2
2.0	1.2	0.4
4.0	2.1	0.6
	B. Flumetralin (Direct Photolysis)	
0.5	0.2	0.1
1.0	0.3	0.2
2.0	0.7	0.5
4.0	1.2	1.0

[a] Estimates of photolysis depth were derived by multiplying the percent recovery of starting material by soil depth when photolysis rates approached zero.

flumetralin was lost. For example, on the low organic Kracaw soil, approximately 40% was photochemically unavailable to a soil depth of 0.25 mm (Figure 3; Table II).

Pesticide sorption on natural surfaces has been reported to suppress various fate processes including photolysis (Yokley et al., 1986; Zepp and Schlotzhauer, 1981). Yokley and co-workers have observed that photolysis rates of polynuclear aromatic hydrocarbons (PAHs) are appreciably slowed on coal fly ashes that are relatively high in carbon and iron content. They proposed that the more porous and darker ashes provided an inner filter protecting the sorbed chemical from phototransformation. Further evidence for an inner filter effect was forwarded by Zepp and Schlotzhauer (1981). These researchers found that sorption of DDE on suspended sediments appreciably inhibited photolysis over extended time periods presumably from time-dependent chemical migration into sediment microenvironments. Because of the strong adsorptive properties of soil, it is reasonable to expect that similar types of microenvironmental interactions may be involved in protecting susceptibile chemicals from photolysis on the sunlight-exposed surface.

Even though mean photolysis depth estimates were not found to be independent, calculated values were generally found to closely agree with reported light penetration measurements (Table VI). The averages of the mean photolysis depth values for all soils and at all soil depths were 0.32 and 0.23 nm for outdoor and laboratory (FS40) irradiations, respectively. The higher direct photolysis depth estimates for outdoor exposures were not surprising in view of possibly greater photochemical availability of the flumetralin from surface disturbances caused by wind and greater surface temperatures that could possibly increase chemical transport to the exposed surface.

Table VI. Average Mean Photolysis Depths (mm) of All Soils Examined

photolysis	soil designation Kracaw	M.S.F.	Montana Grain	Chico Orchard	mean depth
		Direct			
outdoor	0.38	0.13	0.20	0.60	0.32
indoor	0.17	0.15	0.17	0.45	0.23
		Indirect			
outdoor	0.40	0.52	0.50	1.05	0.62
indoor	0.25	0.27	0.25	0.35	0.28

Flumetralin absorbs sunlight over a wider wavelength range (300–500 nm) than most pesticides. Since humic substances have higher extinction coefficients in the shorter wavelengths, the penetration of light into soils is likely greater for the longer sunlight wavelengths. Therefore, the mean photolysis depths determined for flumetralin may be greater than for compounds absorbing only the short-ultraviolet sunlight wavelengths. For example, preliminary data (not shown) indicate that octachlorodibenzo-*p*-dioxin has a more shallow photolysis depth than flumetralin when irradiated simultaneously on soils.

The role organic matter plays in direct photolysis of surface-exposed soils is not clear. Although sunlight and laboratory photolysis depth estimates of soil from the Chico Orchard area were higher than optical thickness measurements for this soil (Table V; Figure 6), steeper thermal gradients may exist on the surface of this very dark soil, enhancing chemical transport. No simple relation between organic content among soils and photolysis rates was observed.

Photosensitized Depth Dependence. The thioether pesticide disulfoton was selected to evaluate the depth dependence of indirect photolysis at the soil surface. Disulfoton does not absorb or undergo direct photolysis in the sunlight spectrum. On soils, however, photosensitized oxidation to its corresponding sulfoxide is rapid.

Over half of a surface-applied concentration of disulfoton was photooxidized in 1–4 days on sunlight-exposed soils (Gohre and Miller, 1986). Although its vapor pressure is moderately high (1×10^{-4} mmHg at 25 °C), photooxidation to the sulfoxide accounted for over 71% of the parent molecule, indicating that volatilization was not a major dissipation process for loss of the compound from soils.

For the four sunlight-exposed soils containing disulfoton, the mean depths for photosensitized oxidations were consistently greater than the simultaneously measured mean depths of photolysis for flumetralin (Figures 7–10; Tables II–V). The averages of the mean estimated photolysis depth among the four soils were 0.28 and 0.72 mm, for laboratory and sunlight-exposed samples, respectively.

A general pattern of disulfoton loss corresponding to soil depth was observed for sunlight-exposed soils from the Kracaws, Main Station Farm, and Montana Grain areas (Figures 7–9; Tables II–IV). On soil from Chico area, however, a depth-dependent relationship was not observed. On this soil, disulfoton was appreciably photooxidized at all soil depths except for that fraction that appeared to be photochemically unavailable (Figure 10).

In the presence of sunlight, singlet oxygen is formed at environmentally significant rates on the soil surface (Gohre and Miller, 1985). The observed greater mean photolysis depth on soil from the Chico area and the other

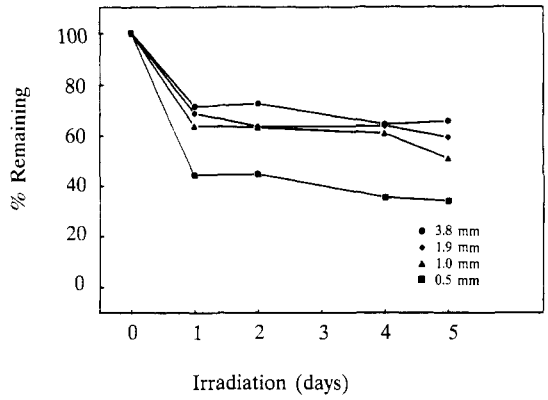

Figure 7. Sunlight photolysis of disulfoton on various thicknesses of Kracaw soil.

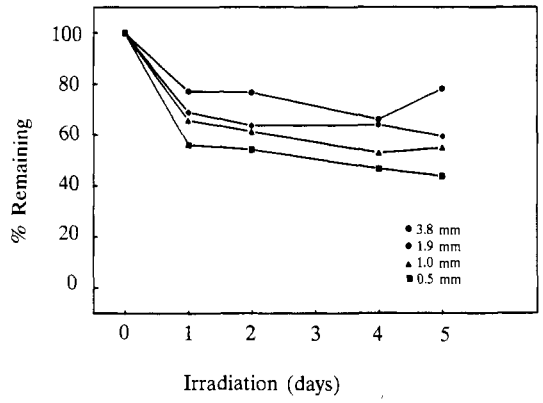

Figure 8. Sunlight photolysis of disulfoton on various thicknesses of Main Station Farm soil.

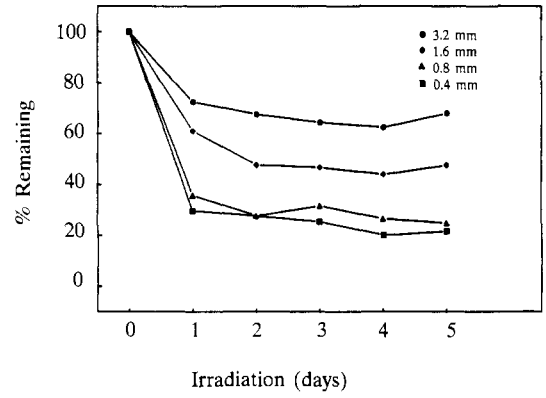

Figure 9. Sunlight photolysis of disulfoton on various thicknesses of Montana soil.

examined soils may be possibly due to penetration of this oxidant to depths greater than the optical depth of soils.

In the vapor phase, the half-life of singlet oxygen can be estimated from the rate constant for deactivation of $^1O_2$, 3.4×10^{-19} cm^3/molecule·s (Penzhorn et al., 1974). At 25 °C, the half-life for singlet oxygen is 0.083 s. The diffusion coefficient of oxygen (D_O) at ambient temperature is 0.207 cm/s (Glinski and Stepniewski, 1985). The mean square distance x^2 diffused by an oxygen molecule in time t can be calculated as follows: $x^2 = 2D_O t$.

On the basis of this reported half-life, the mean diffusion distance of singlet oxygen in air over 1 half-life will be approximately 1.8 mm at 25 °C. The vertical penetration of this oxidant in soils will depend, however, on the degree of compaction, moisture content, and temperature at the sunlight-exposed surface. In agricultural soils, the diffusion coefficient is reduced by a factor of 0.02–0.5 (Glinski and Stepniewski, 1985). On dry porous soil surfaces, singlet oxygen can potentially diffuse to depths approaching 1 mm.

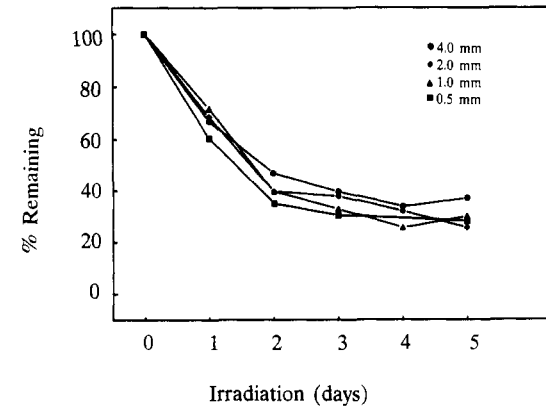

Figure 10. Sunlight photolysis of disulfoton on various thicknesses of Chico soil.

The observed enhancement of indirect photolysis depths in relation to direct photolysis may also be attributable to differences in chemical volatility of pesticides used in this study; disulfoton is substantially more volatile than flumetralin. Although loss in dark controls was negligible among all soils in this study, greater disulfoton availability in relation to flumetralin on the exposed surface could be considerable and, in part, account for the observed enhancement of indirect photolysis to greater soil depths.

CONCLUSIONS

The results from this study support two observations concerning the photochemical fate of pesticides on the soil surface. First, the vertical depth of direct photolysis on the soil surface will be restricted to a region of approximately 0.2–0.3 mm. This observation is consistent with reported values of soil optical depth. Ultraviolet light was found to be greater than 90% attenuated in the top 0.2 mm of the profile. Second, the photooxidation of disulfoton was found to occur appreciably deeper than the optical depth. Mean indirect photolysis depths were reported to be greater than 0.7 mm for outdoor exposures. This observed depth value is conservative when compared to calculated values of mean singlet oxygen diffusion in the vapor phase. In the aerated, unsaturated soil environment, penetration of singlet oxygen to soil depths greater than 2 mm is possible. The depth to which this oxidant vertically diffuses under environmental conditions will depend on moisture content, soil porosity, and steepness of thermal gradients on the sunlight-exposed soil interface. The reported differences in direct and indirect photolysis depths may also to a certain degree reflect differences in chemical volatility of the two pesticides used in this study.

ACKNOWLEDGMENT

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Immobilization of the Serine Protease from *Thermomonospora fusca* YX

Robert D. Johnson,[†] Todd W. Gusek, and John E. Kinsella*

Institute of Food Science, Stocking Hall, Cornell University, Ithaca, New York 14853

The heat-stable serine protease from *Thermomonospora fusca* strain YX was immobilized by cyanogen bromide coupling to Sepharose-4B. The immobilized protease hydrolyzed low molecular weight substrates in accordance with Michaelis–Menton kinetics at 25 °C, while at 65 °C diffusion appeared to be rate-limiting. Immobilization of the protease increased its half-life at 85 °C, by a factor of 3 at pH 6.25 and by a factor of 5 at pH 8.50. A packed column of the immobilized protease efficiently hydrolyzed both bovine serum albumin and β-lactoglobulin. Hydrolysis was monitored by an acid precipitation method as well as colorimetric analysis of terminal amino groups.

The immobilization of proteases from different sources has been widely studied in recent years (Clark and Bailey, 1983; Kumakura et al., 1983; Nakanishi et al., 1985; Skachova and Kucera, 1983). Few reports have addressed the successful immobilization of heat-stable proteases, however, despite the potential benefit of this technology to industry (Cowan and Daniel, 1982; Hultin, 1983).

A thermostable alkaline protease was recently purified from *Thermomonospora fusca* strain YX (Gusek and Kinsella, 1987). The novel protease may be applied to the production of protein hydrolysates for food and medical applications (Gusek and Kinsella, 1988). An immobilized form of the protease in a packed-bed reactor would facilitate the production of a uniform protein hydrolysate, preclude protease autolysis, and circumvent the need to recover the protease from the product eluate. Immobilization of the enzyme to a polysaccharide support using cyanogen bromide was selected because this procedure is amenable to proteins possessing few lysine groups (Axen et al., 1967; Stolzenbach and Kaplan, 1976). The *T. fusca* protease contains a single lysine residue (Kristjansson, 1988).

The objective of this research was to examine the effect of CNBr immobilization on the kinetics and thermostability of the protease from *T. fusca* and the evaluate the proteolytic and esterolytic activity of the immobilized enzyme toward various substrates.

REAGENTS

Sepharose-4B, cyanogen bromide activated Sepharose-4B, *N*-succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine *p*-nitroanilide (SAAPF-pNA), bovine serum albumin (BSA), ovalbumin grade V, equine skeletal myoglobin, hen egg white lysozyme, and casein (sodium salt) were purchased from Sigma Chemical Co., St. Louis, MO. Trinitrobenzenesulfonic acid (TNBS) was purchased from the Aldrich Chemical Co., Milwaukee, WI. Buffer salts, glycerol, formic acid, trichloroacetic acid (TCA), and diethylamine were purchased from Mallinckrodt Inc., Paris, KY. Sephadex G-25 and CM-Sephadex C-50 resins were purchased from Pharmacia Inc., Piscataway, NJ. β-Lactoglobulin (βLG) was purified in this laboratory by the method of Armstrong et al. (1967).

[†] Current address: Department of Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.

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