Table III. Nitrocresol Recoveries from *T. viride* Cultures

	% nitrocresol recd:[a] addn at day				
expt no.	0	3	5	7	14 (ee)
11	55.3[b]	57.0	70.3		102.6[c]
12	63.8	63.3	74.6	77.2	99.0

[a] No cleanup of extracts; nitrocresol silylated for analysis. [b] All values result from triplicate cultures and analyses. [c] After 2 h equilibration, represents extraction efficiencies.

difference between the two compounds was nowhere near the several orders of magnitude difference observed (Zitko and Cunningham, 1974) with alkaline hydrolysis.

The above observations would account for the hypothesis that (i) enzymatic hydrolysis of the test compounds proceeds throughout the incubation period and (ii) during the period of growth (i.e., days 1–4) the test compounds and/or the nitrocresol formed are cometabolized by the fungus.

Rather than carrying out further delayed addition experiments with the oxon, it was decided to incubate *T. viride* cultures directly with 3-methyl-4-nitrophenol. Addition of this compound at day zero (i.e., with inoculation) and at days 3, 5, and 7 gave results as summarized in Table III. It is clear that a substantial portion of the nitrocresol "disappears" during the first few days and that progressively higher nitrocresol recoveries are obtained when this compound is added after day 4. This suggests that the missing material in experiments 1, 2, 3a, and 4 is primarily due to cometabolism of the nitrophenol after hydrolysis rather than to direct consumption of fenitrothion. The complete absence of any other known metabolites or other unidentified compounds in the extracts of experiments 11 and 12 is a strong indication that a substantial portion of the nitrocresol is consumed by cometabolism.

These results complement work done on malathion and parathion as reviewed extensively by Mulla et al. (1981). These authors cite several reports of microorganisms, primarily bacteria, utilizing these thiophosphate pesticides as a sole course of carbon, phosphorus, and nitrogen. The present results obtained with fenitrothion and a fungus, and indicating hydrolysis as well as cometabolism in the presence of abundant alternate nutrients, would appear to be more relevant from an environmental point of view.

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Photooxidation of Thioether Pesticides on Soil Surfaces

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Four thioether-containing pesticides undergo photosensitized transformation to the corresponding sulfoxides when sorbed on soil surfaces and exposed to sunlight. The pesticides examined were disulfoton, methiocarb, butocarboxim, and fenthion. Disulfoton underwent the most rapid loss on the three soils examined, and half of the originally applied pesticide was degraded in 1–4 days. Methiocarb generally degraded the most slowly. The probable oxidant forming the sulfoxides is singlet oxygen, although other sensitized oxidation pathways are also possible. The production of the sulfoxides was demonstrated not to be due to metabolic processes. The lowest organic content soil (0.79%) gave the most rapid degradation rates. The rates of conversion of these compounds to the sulfoxides were sufficiently rapid that photooxidation is likely an important transformation pathway of these compounds on soil surfaces.

INTRODUCTION

The production of singlet oxygen ($^1O_2$) on irradiated soil surfaces using chemical traps has previously been demonstrated (Gohre and Miller, 1983). The rapid degradation of the parent chemicals and generation of singlet oxygen products suggest that singlet oxygen may be a important oxidant on irradiated soil surfaces and may play a significant role in the transformation of electron-rich xenobiotics exposed to sunlight on the surface of soils.

Many sulfur-containing pesticides in use today have thioether or thioketone groups susceptible to singlet oxygen oxidation (Tamagaki and Hotta, 1980; Arjunan et al., 1984; Liang et al., 1983). Studies have examined the photochemistry of thioether-containing pesticides on silica gel and in aqueous solution, organic solution, plant, and glass

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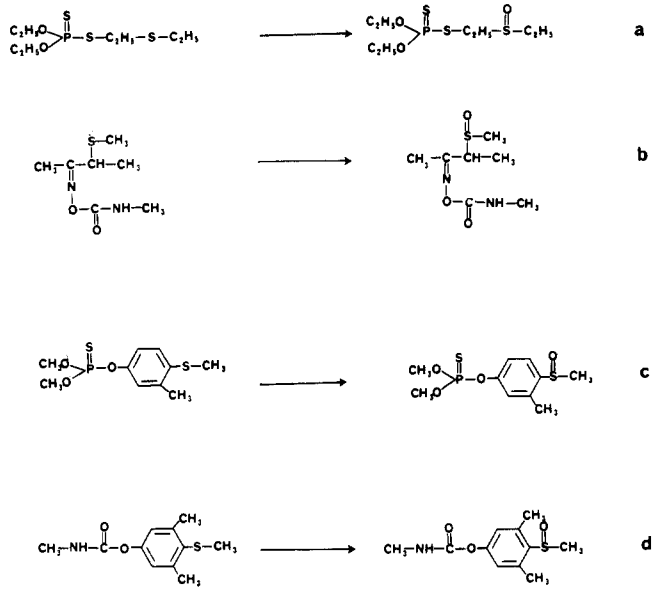

Figure 1. Sunlight photolysis of butocarboxim (b), fenthion (c), and methiocarb (d) and their sulfoxide analogues.

surfaces. Murai (1981) found that xanthone sensitizes the photooxidation of propaphos to its sulfoxide and sulfone in sunlight on silica gel. Zepp and co-workers (1981) concluded that the photosensitized conversion of disulfoton to the sulfoxide was due to dissolved humic substances abundant in natural waters producing singlet oxygen. The photolysis of the thioether insecticide Mesurol (Abdel-Wahab et al., 1966) on glass and bean plant surfaces produced the sulfoxide and sulfone under 254-nm light but not under 366-nm light. Ivie and Bull (1976) found that sunlight irradiation of the thioether organophosphorus pesticide (BAY NTN 9306) on cotton foliage, glass surfaces, and water solutions was rapid in each system, with conversion to the sulfoxide and sulfone. Fujii and co-workers (1979) examined the photooxidation of propaphos on silica gel, on glass surfaces, and in water in the presence of sunlight. Degradation was rapid in all cases, with the major photoreactions being oxidation of the thioether and photohydrolysis.

The present work examines the soil-sensitized photolysis of four thioether pesticides susceptible to singlet oxygen reactions. Disulfoton, fenthion, methiocarb, and butocarboxim were exposed to sunlight to determine whether each undergoes photochemical conversion to the sulfoxide when sorbed on soil surfaces (Figure 1). The purpose of this work was to establish whether the soil-sensitized photolysis of these sulfur-containing pesticides occurs at environmentally important rates and determine whether the photoproducts are generated in significant yields.

MATERIALS AND METHODS

Materials. All solvents (Burdick and Jackson) were distilled in glass. Fenthion (99.6%) was provided by Chemagro Co. Disulfoton (98.6%), disulfoton sulfoxide (>95%), disulfoton sulfone (>95%), methiocarb (98.7%), methiocarb sulfoxide, and methiocarb sulfone were provided by Mobay Chemical Co. Butocarboxim (99%), butocarboxim sulfoxide (99%), and butocarboxim sulfone (99%) were obtained from Wacker Chemical Co. All organic reagents were checked for purity by gas–liquid chromatography (GLC) or high-pressure liquid chromatography (HPLC).

Pesticide Photolysis on Soils. Selected soil properties are presented in Table I. Preparation of the soils was described previously (Gohre and Miller, 1983). Sterilized soils were prepared by autoclaving at 16 psi and 121 °C for 1 and 0.5 h successively. Two different application methods were used to assess pesticide photodegradation on soil surfaces. Soil (10 g) was saturated with distilled water, placed in uncovered Petri dishes (10-cm diameter), and then allowed to dry outdoors. This method gave a firm soil surface that limited wind erosion losses when the dishes were exposed outdoors. A methanol or methylene chloride solution of each pesticide was applied to the soil surface (50–200 ppm) with a syringe, and the samples were exposed outdoors. After the exposure was complete, the soil was transferred into a 100-mL volumetric flask and the pesticide residues were extracted into methanol (20 mL). The methanol was separated from the bulk soil by centrifugation and the supernatant filtered through a 0.45-μm Gelman filter. The supernatant was transferred to a 25-mL volumetric flask that was brought to volume and refrigerated prior to analysis.

Table I. Soil Properties

soil design[a]	% org matter	pH	% clay	% silt	% sand
Kracaws	0.79	7.5	8	47	48
M.S.F.	1.97	7.2	14	32	54
Valley road	3.49	5.7	13	17	70
Richvale ricefield	5.19	4.5	15	35	50
Chico	6.31	6.5	14	31	55

[a] Soils were collected from the following locations: Kracaws, 40 km north of Winnemucca, NV; M.S.F., University of Nevada research farm; Valley road, field station at the University of Nevada, Reno; Chico, walnut orchard 4 km west of Chico, CA; Richvale ricefield, near Richvale, CA.

In the second method, pesticide was sprayed onto soil (50–200 ppm) in methylene chloride with the use of an atomizer. The soil was mixed, then divided into replicate samples (1.75 g), placed in 50-mL Kimax volumetric flasks, and stoppered with Teflon septa. Two samples were analyzed immediately as zero time standards. After completion of sunlight exposure, 3.0 mL of methanol was added to the flask, and the sample was analyzed as described above.

All photooxidations of pesticides on soil were conducted outdoors and exposed to sunlight.

Pesticide Identification and Quantitation. Quantitation of the pesticides was accomplished by HPLC, GLC, or GC–MS. The HPLC analyses were performed on a C_{18} reversed-phase Altex 5-μm column (15 cm × 4.6 mm i.d.) using various mixtures of methanol and water as the mobile phase. The HPLC utilized was a solvent-programmable LDC system with variable-wavelength detection. Quantitations of pesticide sulfoxides were done by HPLC since their thermal instability gave unsatisfactory GLC peaks. The sulfoxides and sulfones of disulfoton, fenthion, methiocarb, and butocarboxim were identified by HPLC cochromatography with known standards or by their mass spectra. The gas chromatograph was an HP-5830 equipped with a flame ionization detector and a 1.5-m glass column packed with 3% OV-210 on 100/120-mesh Gas Chrom Q. The mass spectrometer was a Finnegan 4023 GC–MS equipped with the same column.

RESULTS

Photooxidations of the dialkyl thioether pesticide disulfoton and the alkyl aryl thioether pesticide fenthion were compared on four soils with varying organic content. These compounds were atomized onto the soils and exposed to sunlight in 50-mL volumetric flasks. Although disulfoton does not absorb in the sunlight spectrum, it was rapidly degraded at similar rates on the four soils. Half of the original concentration in each case was lost in ap-

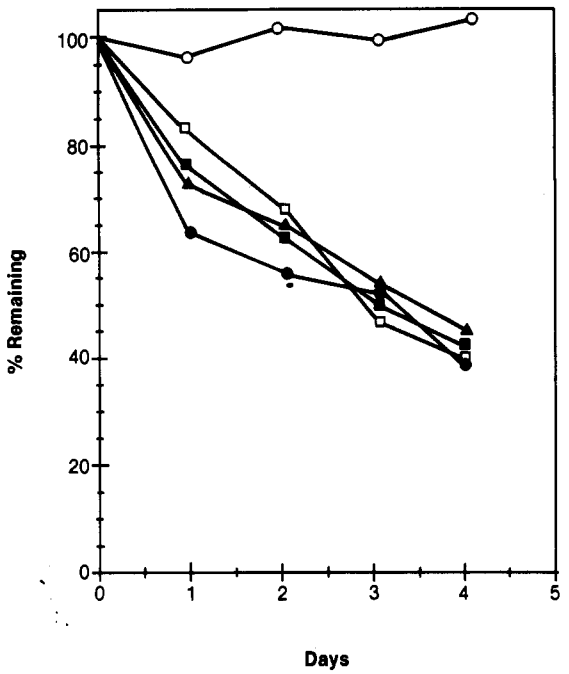

Figure 2. Photosensitized loss of disulfoton from four soils and a bare flask exposed outdoors in 50-mL Kimax volumetric flasks (start 10/21/83). The soils and dark recoveries (aluminum foil covered flasks) after 4 days were as follows: bare flask (○), 101%; MSF (□), 102%; Chico (▲), 91.5% Richvale (■), 100.5%; Kracaws (●) 87.4%.

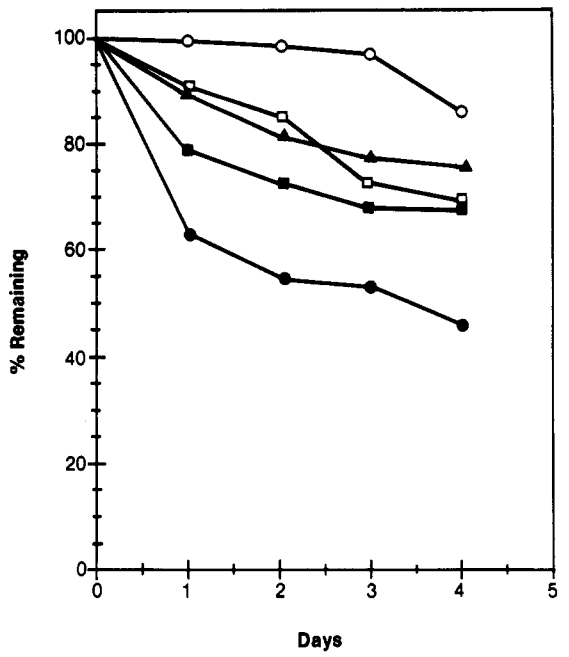

Figure 3. Photosensitized loss of fenthion from four soils and a bare flask exposed outdoors in 50-mL Kimax volumetric flasks (start 5/8/84). The soils and dark recoveries (aluminum foil covered flasks) were as follows: bare flask (○), 94.1%; Richvale (▲), 94.1% MSF (□), 97.9%; Chico (■), 84.9%; Kracaws (●), 98.4%.

proximately 3 days (Figure 2). The rates of photolysis generally were not first order and substantially decreased over the course of the irradiations, presumably due to light screening. The primary photoproduct was the sulfoxide, with trace amounts (<5% of sulfoxide yields) of the sulfone observed. The bare-flask photolysis (indicative of direct photolysis) showed minor disulfoton loss with no sulfoxide production. The loss of each pesticide from the dark controls was also negligible during the course of the ex-

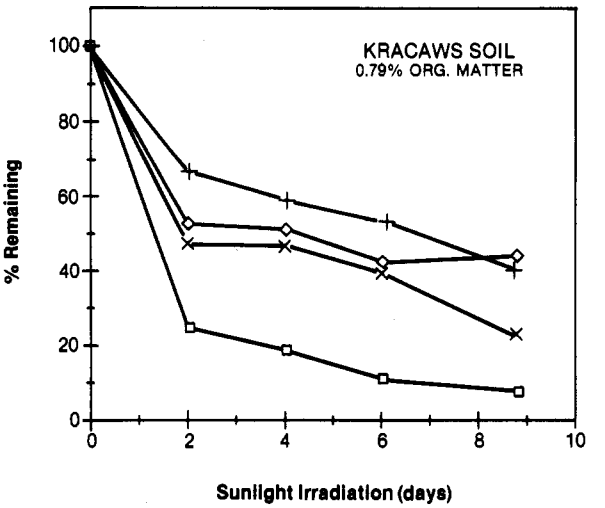

Figure 4. Sunlight photolysis of butocarboxim (◇), methiocarb (+), fenthion (×), and disulfoton (□) on Kracaws soil in 50-mL Kimax volumetric flasks (start 8/27/84).

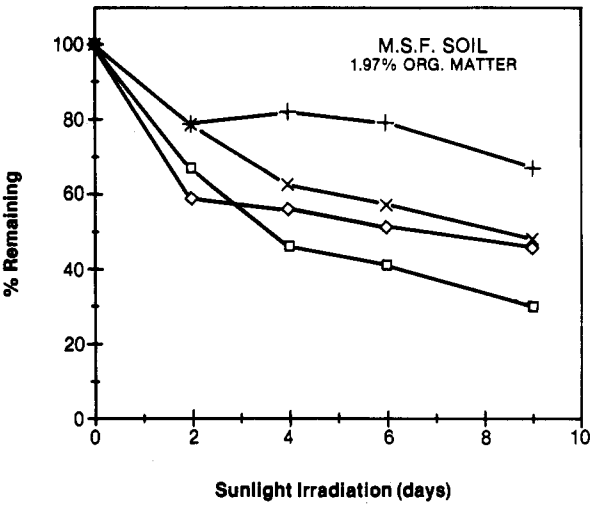

Figure 5. Sunlight photolysis of butocarboxim (◇), methiocarb (+), fenthion (×), and disulfoton (□) on M.S.F. soil in 50 mL Kimax volumetric flasks (start 6/6/84).

periments with no conversion to sulfoxide.

Fenthion was also photodegraded on several soils (Figure 3), although the rates were slower than for disulfoton. The rate of loss was fastest on the lowest organic soil, and the major photoproduct identified on all the soils was the sulfoxide. Rates of fenthion degradation in bare flasks and dark controls were significantly slower with no production of the sulfoxide. Although a quantitative determination of the relative photolysis rates of disulfoton and fenthion cannot be made because of seasonal differences in sunlight intensity, the sunlight photolysis rate of disulfoton is more rapid since it degraded more rapidly in October when sunlight intensity was lower than in May, when fenthion was exposed (Zepp and Cline, 1977).

The sunlight photooxidation of four thioether-containing pesticides was evaluated on three representative soils, M.S.F., Chico, and Kracaws, under similar conditions to distinguish differences in reactivity between the pesticides on the same soil. The pesticides were atomized onto the soils that were then placed in 50-mL volumetric flasks and exposed to sunlight. Methiocarb degraded the slowest and disulfoton degraded the most rapidly on each of the soils (Figures 4–6). Significantly, the most rapid rates of degradation occurred on the lowest organic carbon soil (Kracaws). The major degradation product for all the sulfides was the sulfoxide analogues.

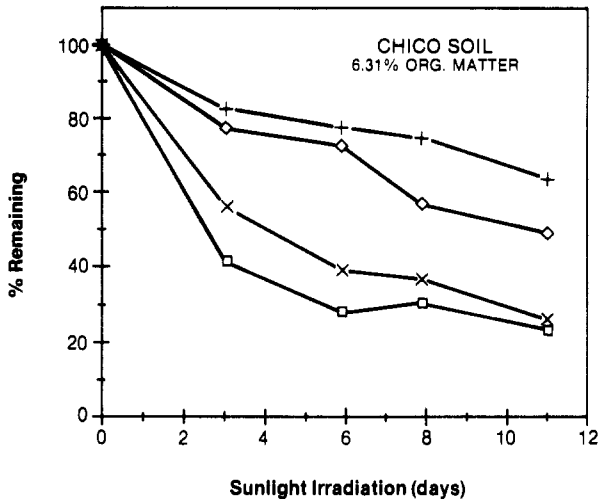

Figure 6. Sunlight photolysis of butocarboxim (◊), methiocarb (+), fenthion (×), and disulfoton (□) on Chico soil in 50-mL Kimax volumetric flasks (start 7/17/84).

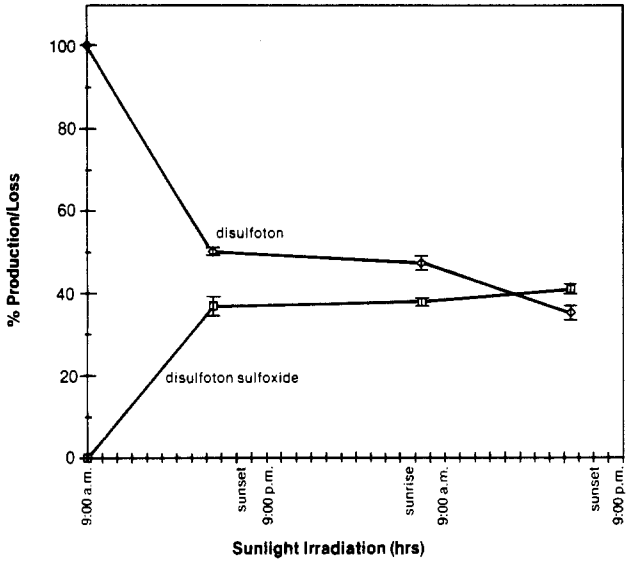

Figure 7. Sunlight photolysis of disulfoton spotted on M.S.F. soil on open Petri dishes (start 8/21/84).

Sunlight photooxidation of disulfoton and methiocarb was also conducted in uncovered Petri dishes to better assess the above results in a situation similar to field conditions where volatilization will potentially contribute to loss of the pesticides. The two thioether compounds were applied to soil in uncovered Petri dishes with a syringe and exposed to sunlight on Valley Road soil over a 24-h period. The results of these experiments are presented in Figures 7 and 8. Loss was most rapid with disulfoton and slowest with methiocarb, as expected. The time for loss of half of the original concentration was approximately 1 day less than in the borosilicate volumetric flasks. This is noteworthy since the Petri dish experiments were done later in the year than the closed-flask experiments and at a similar time as the Kracaws soil experiments. Degradation of the parent pesticides and conversions to the sulfoxide analogues occurred during sunlight hours. The overall yields of disulfoton sulfoxide and fenthion sulfoxide were 71% and 63%, respectively, and indicate that, under these conditions, the primary loss of the parent thioether was due to phototransformation to sulfoxides and not volatilization. Volatilization may, however, be substantially greater under conditions where the soil surface is wet.

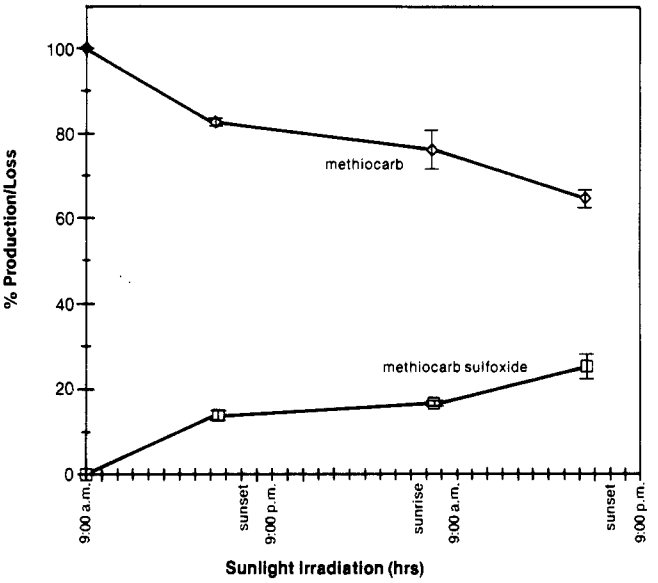

Figure 8. Sunlight photolysis of methiocarb spotted on M.S.F. soil on open Petri dishes (start 8/21/84).

Table II. Photosensitized Oxidation of Disulfoton on Sterilized vs. Unsterilized Soil

	% recovery[a]			
	unsterilized soil		sterilized soil	
soil type	disulfoton	disulfoton sulfoxide	disulfoton	disulfoton sulfoxide
Kracaws[b]	32	50	13	57
Richvale[b]	26	37	32	34
Valley road[b,c]	21	54	22	46

[a] Percent recoveries are based on the moles of starting material. [b] Seven-day sunlight photolysis from 7/4/84 to 7/11/84 in closed 50-ml Kimax volumetric flasks in air; average of three replicates. [c] This soil was collected from the field and used immediately.

Since soil microorganisms are also able to convert thioethers to sulfoxides and sulfones by cytochrome P-450 and FAD-dependent monooxygenases, their contributions to the soil surface oxidations could conceivably contribute to the oxidations. The microbial conversion of the thioether pesticides phorate and fenthion has been documented (Menzie, 1969; Ahmed and Casida, 1958). Sunlight photolysis of disulfoton was conducted in 50-mL volumetric flasks comparing the relative activities of sterile and unsterile soil. The results showed that microbial metabolism (Table II) was not involved in the observed disulfoton loss and the resulting sulfoxide production.

DISCUSSION

Sunlight irradiation of four thioether pesticides sorbed on soil surfaces resulted in loss of each parent compound with predominant conversion to the sulfoxide analogues. Only trace amounts of the sulfones were detected. The oxidations were primarily sensitized, since direct photolysis in bare flasks, if observed, was substantially slower. Additionally, there was no difference in photolytic activity between sterile and nonsterile soils. The rapid loss of these pesticides and conversion to the sulfoxides suggests that photosensitization is likely an important process in the transformation of these chemicals under field conditions. Singlet oxygen is the likely reactant, since it is produced on both irradiated soils (Gohre and Miller, 1983) and also irradiated inorganic metal oxides such as silica gel and alumina (Gohre and Miller, 1985).

Disulfoton was rapidly photooxidized, while the aryl sulfides were more slowly degraded. The reason for the marked difference in reactivity between diethyl and aryl

sulfides is not well understood, although steric effects have been suggested (Monroe, 1979). Reaction rates for a series of alkyl sulfides with singlet oxygen show a marked decrease as the carbons α to sulfur become more substituted. For simple sulfides this reactivity to singlet oxygen in organic solutions is ethyl sulfide > 2-butylpropyl sulfide > *tert*-butyl ethyl sulfide > methyl phenyl sulfide (Wilkinson and Brummer, 1981). Using the above photoreactivities, the expected singlet oxygen reactivity would be disulfoton > butocarboxim > fenthion > methiocarb. Therefore, the rapid photosensitization of disulfoton to its sulfoxide was expected. Methiocarb is expected to be fairly unreactive due to steric crowding. Fenthion was more reactive to singlet oxygen than expected from this simple comparison, perhaps due to substituent effects on the aromatic ring.

Photosensitized oxidation of several classes of other pesticides has previously been demonstrated in other systems, including pyrethrins, thiabendazole, pyridine base fungicides, and other thioether-containing pesticides. Thus, these types of reactions are probably important for transformation of agricultural pesticides susceptible to singlet oxygen oxidation. The natural pyrethrins and the early synthetic pyrethroids contain furan and isobutenyl groups that are easily oxidized by singlet oxygen (Ruzo, 1983). Though potent insecticides, this inherent photoinstability has lead to the synthesis of more photostable synthetic pyrethroids. The fungicide thiabendazole was shown to be photodegraded in 1% methanolic solutions containing methylene blue (Mahran et al., 1983). Although degradation was attributed to singlet oxygen, potential methylene blue mediated electron-transfer reactions were not considered (Manring et al., 1980). In addition, rose bengal sensitized photooxidation of the pyridine base fungicides ethirimol, dimethirimol, and 2-(dimethylamino)-5,6-dimethylpyrimidin-4-ol in aqueous solution results in rapid oxidation of those compounds (Harkness and Wells, 1981; Dixon and Wells, 1983).

Although direct photolysis reactions on soil surfaces are often slowed relative to solution photolysis (Miller and Crosby, 1983), indirect reactions appear to be enhanced. Particularly for those pesticides susceptible to singlet oxygen reactions, indirect photolysis will likely be an important transformation pathway for surface-applied materials. Further work is ongoing to examine other classes of compounds and also determine the depth dependence for photosensitized oxidation on soils.

Registry No. **a**, 298-04-4; **a** (sulfoxide), 2497-07-6; **b**, 34681-10-2; **b** (sulfoxide), 34681-24-8; **c**, 55-38-9; **c** (sulfoxide), 3761-41-9; **d**, 2032-65-7; **d** (sulfoxide), 2635-10-1.

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