Degradation of [14C]Tebupirimphos under Anaerobic Aquatic Conditions

Premjit P. Halarnkar,* William M. Leimkuehler, Debra L. Green, and Vickie A. Marlow

Bayer Corporation, Agriculture Division, Environmental Research, 17745 South Metcalf,
Stilwell, Kansas 66085

An anaerobic aquatic metabolism study with [*pyrimidinyl*-2-14C]tebupirimphos at a concentration of 2 ppm was conducted using a sandy loam flooded with pond water at a constant 22 °C. Over the course of the study, extractable residues from water decreased from 56.9% at day 0 to 42.6% of the applied radioactivity at day 127. Extractable residues from soil decreased from 38.3% at day 0 to 30.7% at day 127. Nonextractable residues in water and soil at the end of day 127 were 22.3% and 7.4%, respectively. Volatile residues never exceeded 0.1% of the applied radioactivity during the entire period of incubation. The parent concentration declined from 93.5% at day 0 to 51.1% at day 127. The half-life of tebupirimphos under anaerobic aquatic conditions was calculated to be 194 days. The degradates formed were desisopropyltebupirimphos, desethyltebupirimphos, *tert*-butylhydroxypyrimidine, and the *S*-ethyl isomer of isopropyltebupirimphos. Desisopropyltebupirimphos and *tert*-butylhydroxypyrimidine were the major products (>10%), whereas desethyltebupirimphos and the *S*-ethyl isomer of isopropyltebupirimphos never exceeded 3% of the applied radioactivity during the entire period of incubation.

Keywords: *Anaerobic; aquatic; metabolism; tebupirimphos*

INTRODUCTION

To adequately evaluate the potential risk associated with the use of a pesticide, it is important to know the fate of a pesticide in the environment. This involves understanding the nature and extent of pesticide residues in water and hydrosoil. Here we report the results of a study conducted under anaerobic aquatic conditions. Anaerobic conditions can exist in soils, sediments, and water-logged environment. We studied the metabolism of [14C]tebupirimphos, an organophosphate insecticide developed by Bayer Corp., under anaerobic aquatic conditions. Tebupirimphos is the major ingredient in AZTEC, recently registered for use on corn, and it is applied at planting as a granular material. Since anaerobic degradation is only one of many possible dissipation routes, the overall fate of tebupirimphos is also reviewed. This is important in order to allow the reader to understand how anaerobic aquatic degradation fits within the overall fate profile.

EXPERIMENTAL PROCEDURES

Chemicals. The structure of [*pyrimidinyl*-2-14C]tebupirimphos [*O*-ethyl *O*-(2-methylethyl) *O*-(2-(1,1-dimethylethyl)-pyrimidin-5-yl) phosphorothioate] (specific activity = 21.62 mCi/mmol) is shown below, with the 14C-labeled position marked by an asterisk.
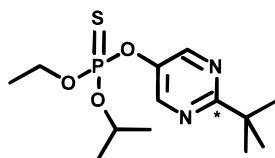
Soil. A fresh sample of sandy loam (Shipshe series, obtained from Howe, IN) was air-dried overnight and sieved through a 2-mm mesh sieve. The soil moisture content was

determined, and accordingly, 15-g aliquots of soil (dry weight) were weighed into 250-mL Erlenmeyer flasks. Pond water was obtained from Howe, IN, and was enriched with glucose (5.0 mg/mL) and calcium nitrate (8.4 mg/mL) to ensure microbial growth. The soil in the incubation flasks was flooded with 150 mL of enriched water. The flasks were closed using a custom-made glass stopper equipped with an adapter to pass nitrogen through the test system. The headspace was purged with nitrogen (600 mL/min) for 30 s immediately after each flask was sealed with the laboratory film (Parafilm). The flasks were then wrapped with aluminum foil and were kept static at 22 ± 1 °C for 30 days to establish anaerobic conditions.

Test for Anaerobic Conditions. After 30 days of incubation, but prior to treatment, a few sampling flasks were removed from the incubator. These flasks were opened in a closed glovebox which was filled with nitrogen, and redox potential, dissolved oxygen, and pH were measured. The redox potential and pH were measured by a digital pH/mV/ORP meter (Cole Palmer), whereas dissolved oxygen was recorded using a YSI model 58 oxygen meter, equipped with a YSI model 5739 dissolved oxygen probe. Additional control flasks were subsequently used at every sampling interval to record redox potential, dissolved oxygen, and pH. During the entire incubation period of 127 days, the redox potential remained negative (values ranged from −10 to −527 mV), indicating an anaerobic environment in the system.

Preparation of Treatment Solution and Treatment. The treatment solution was prepared by removing 1 mL of [14C]tebupirimphos, evaporating the solvent (benzene), and redissolving the material in 2 mL of acetonitrile. Prior to treatment, radiochemical purity of the treatment solution was determined by high-pressure liquid chromatography (HPLC) and found to be 98.3%. To each flask, 56 μL of treatment solution, containing 300 μg of tebupirimphos, was added on the top of the water without shaking. This resulted in an initial theoretical concentration of 2 ppm in water. The flasks were immediately closed with stoppers, purged with nitrogen at a rate of 600 mL/min for 30 s, sealed with laboratory film (Parafilm), and returned to the incubator.

Sampling and Extraction. Two samples were analyzed immediately after application of [14C]tebupirimphos (day 0). Subsequently, duplicate samples were analyzed at 7, 14, 28, 59, 92, and 127 days post-treatment. Prior to removing the stoppers from the flasks, the headspace was purged with

* To whom correspondence should be addressed (FAX: 913 433-5389).

2350 *J. Agric. Food Chem.,* Vol. 45, No. 6, 1997

Halarnkar et al.

Table 1. Distribution of [¹⁴C] Residues in the Tebupirimphos Anaerobic Aquatic Degradation Study[a,b]

days post-application	0[c]	7	14	28	59	92	127
volatiles		<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
water							
EtOAc extract	56.9	46.1	47.3	36.9	40.1	42.1	42.6
aqueous phase	1.8	4.2	6.4	10.8	13.5	16.6	22.3
subtotal	58.7	50.3	53.7	47.7	53.6	58.7	64.9
soil							
MeOH extract	38.3	36.9	34.5	43.6	38.5	39.5	30.7
bound	0.1	1.5	2.4	2.6	3.3	4.5	7.4
subtotal	38.4	38.4	36.9	46.2	41.8	44.0	38.1
total	97.1	88.7	90.6	93.9	95.4	102.7	103.0

[a] All residues are reported as percent of applied radioactivity. [b] Results are the mean of two samples per interval. [c] Results are from a single sample.

Table 2. Summary of [¹⁴C] Degradate Distribution in Total Sample (Soil and Water) Extracts[a]

days post-application	0[b]	7	14	28	59	92	127
tebupirimphos	93.5	78.0	77.1	74.8	69.8	67.1	51.1
desisopropyltebupirimphos		3.8	10.8	12.5	14.3	19.4
TBHP[c]	0.8	3.1	2.5	4.1	8.2	14.5	22.2
desethyltebupirimphos			0.3		1.0	1.0	2.6
isopropyltebupirimphos (*S*-ethyl isomer)	0.9	2.0	2.0	1.4	0.5	0.4	
other products[d]			0.1	0.3		0.9	0.4

[a] All residues are reported as percent of applied radioactivity. Results are the mean of two samples per interval. [b] Results are from a single sample. [c] TBHP: *tert*-butylhydroxypyrimidine. [d] These contain several minor unidentified degradates.

nitrogen at a rate of 40 mL/min for 15 min through a series of traps to capture volatile metabolites. The traps contained 30 mL of NaOH (1 M), ethylene glycol, and sulfuric acid (5 M). Triplicate aliquots from each trap were radioassayed by liquid scintillation counting. Prior to separating water and sediment, the pH of the water was recorded. The soil was vacuum filtered (Whatman, no. 41), and the filtrate was extracted with ethyl acetate (2 × 150 mL). The filtered soil was then extracted with methanol (2 × 150 mL) by stirring for 3 h at room temperature. The soil was separated from the methanol by vacuum filtration.

At each step of extraction, triplicate aliquots of ethyl acetate, aqueous phase, and methanol extracts were assayed for radioactivity. The solvents were then evaporated by rotovap (Buchi). Aliquots of filtered solids, before and after extraction, were oxidized and radioassayed.

Samples (extracts) were kept in the freezer (temperature range from −15 to −35 °C) immediately after extraction. Both HPLC and thin-layer chromatography (TLC) analyses were performed within 30 days of extraction. The reference standards were dissolved in methanol and kept in the freezer.

Radiometric Analysis. Radioactive measurements were made using a Packard Tricarb Model 4640 liquid scintillation counter (LSC), equipped with automatic external standardization. Liquid samples were radioassayed by adding 10 mL of Ultima Gold scintillator (Packard) and 2 mL of water. The radioactive value for each sample was an average of triplicate measurements.

Soil samples were combusted in a sample oxidizer (Packard, Model 306), and the released ¹⁴CO₂ was trapped in 6 mL of Carbosorb E (Packard) and 15 mL of PermaFluor E⁺ (Packard). The radioactivity was measured by LSC.

Thin-Layer Chromatography. Extracts were analyzed by TLC using Merck silica gel 60 F-254 plates (normal phase, 0.25 mm, 20 × 20 mm). The ethyl acetate, methanol, and aqueous phase extracts were applied to TLC plates with the appropriate standards and developed in either hexane:acetone (4:1) or chloroform:methanol (3:1). Radioactive zones were detected using a radioactivity TLC-scanner (Raytest Rita 6800) and by exposing the plates to Kodak X-ray film (type XAR-5).

High-Pressure Liquid Chromatography. Quantitative analysis of samples was performed on a Hewlett-Packard 1090 HPLC, equipped with a UV detector (254 nm) and radioactivity monitor (Raytest Ramona 90). A reverse phase ODS column (Phenomenex, Ultrasphere 5-*µ*, 250 × 4.6 mm), equipped with a C-18 guard column, was used for the separation of tebupirimphos and its metabolites. The solvents were acetonitrile and
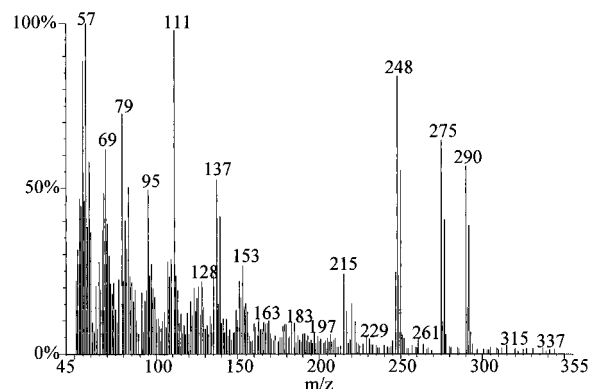
Figure 1. Mass spectrum of the methyl derivative of desisopropyltebupirimphos obtained by GC−MS.

water containing 0.4% acetic acid. The gradient was from 20% acetonitrile to 80% in 20 min, increased to 100% in another 30 min, and then held at 100% for 10 min. The flow rate was 1 mL/min.

Metabolite Purification and Derivatization. Tebupirimphos and its metabolites were purified by TLC and/or HPLC, using the same systems employed for sample extractions. After isolating the peak of interest, HPLC solvent (acetonitrile/water) was evaporated under nitrogen and the material was redissolved in methanol. This solution was passed through small amount of cation exchange resin (Dowex, 50W-X8). The metabolite was eluted with methanol, the solvent volume was reduced to about 100 *µ*L under nitrogen, and then 150 *µ*L of diazomethane solution in diethyl ether was added. The reaction was allowed to proceed for 1 h at room temperature. The ether was then evaporated under nitrogen, and the derivatized samples were further analyzed by HPLC and mass spectrometry.

Mass Spectrometry Analyses. Mass spectral analyses were carried out by liquid chromatography−mass spectrometer thermospray (LC-MS-TSP), using a FINNIGAN-MAT 90 double-focusing mass spectrometer along with a Varian 5040 HPLC. A Regis ODS column (150 × 4.6 mm) was used with methanol and water as solvents. The gradient was 0% to 100% methanol over 30 min, with a flow rate of 0.8 mL/min. Ammonium acetate buffer (0.1 M) was added "post-column" at a rate of 0.5 mL/min. For GC−MS analysis a Hewlett-Packard 5890 gas chromatograph (GC), equipped with a 15-m DB-5 MS
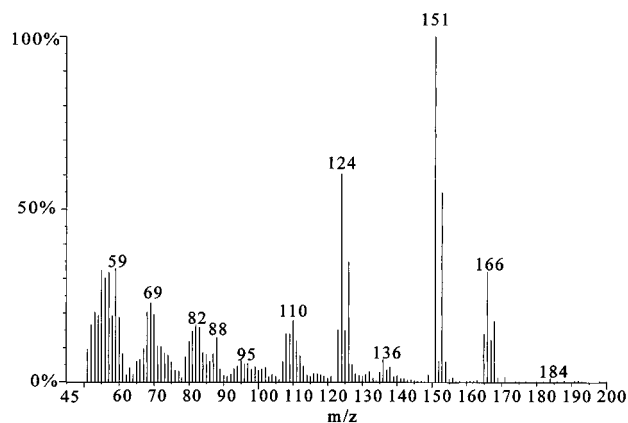
Degradation of [14C]Tebupirimphos

J. Agric. Food Chem., Vol. 45, No. 6, 1997 **2351**

Figure 2. Mass spectrum of the methyl derivative of *tert*-butylhydroxypyrimidine obtained by GC–MS.

(J&W) capillary column (0.25 mm i.d.), was coupled to a Finnigan INCOS 50 quadrapole mass spectrometer. The GC column oven was held at 80 °C for 2 min, then increased from 80 to 250 °C at 15 °C/min, and then held at 250 °C. The injector was set at 250 °C, and helium was the carrier gas (approximate flow of 1 mL/min.). The mass spectrometer ion

source was held at 180 °C, and the mass range from 50 to 400 amu was scanned at 1 s/scan.

RESULTS AND DISCUSSION

Table 1 shows the distribution of [14C] residues in the anaerobic aquatic system. On day 0, radioactive residues from water very readily partitioned into the soil layer. This was probably due to shaking of water and soil before filtration. Residues in the soil were 38.4% on day 0, and this amount increased to 46.2% by day 28. On day 127 the residues were 38.1% in the soil. The remaining residues after methanol extraction were considered as bound residues. The bound residues in the soil increased from 0.1% at day 0 to 7.4% at day 127. Residues in water increased slightly from 58.7% at day 0 to 64.9% at day 127. Nonextractable residues in water increased from 1.8% at day 0 to 22.3% at day 127. The average material balance was 95.9%, with values ranging from 88.7% to 103.0% of the applied radioactivity. The volatiles accounted for less than 0.1% at all sampling intervals.

The degradation of tebupirimphos exhibited first-order degradation kinetics with a half-life of 194 days
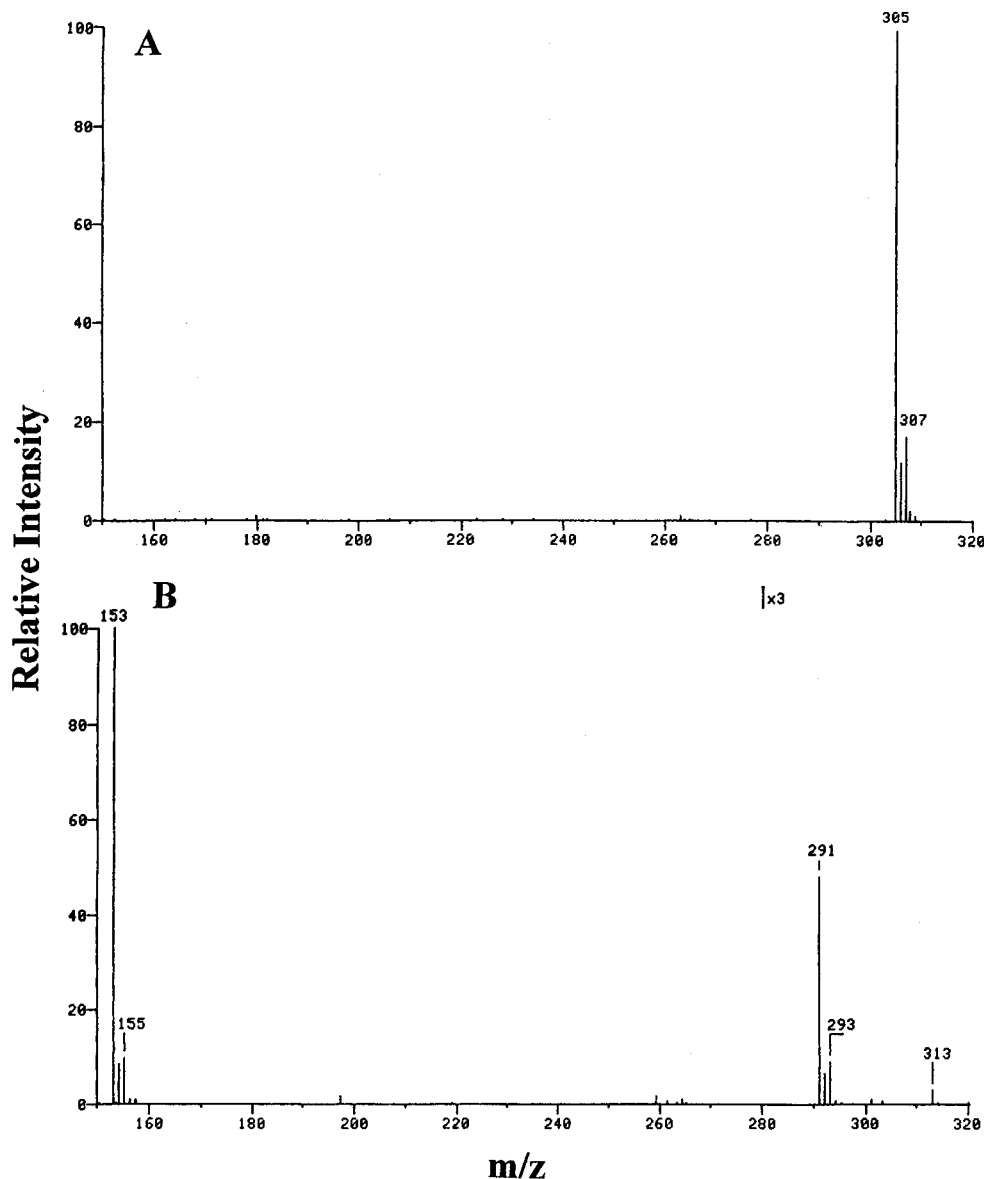

Figure 3. Mass spectra of the *S*-ethyl isomer of isopropyltebupirimphos (A) and desethyltebupirimphos (B) obtained by LC-MS-TSP in the positive-ion mode.
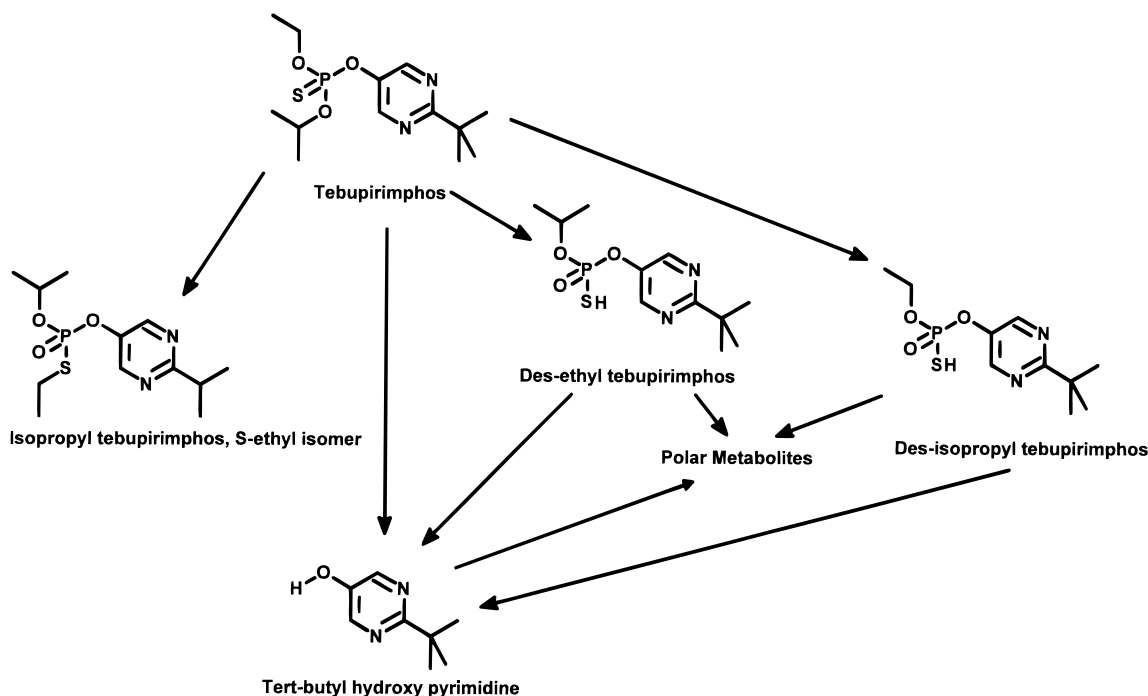
2352 *J. Agric. Food Chem.,* Vol. 45, No. 6, 1997

Halarnkar et al.

Figure 4. Proposed degradation pathway for [^{14}C]tebupirimphos under anaerobic aquatic conditions.

($r^2 = 0.857$), determined by linear regression analysis. Table 2 summarizes the distribution of degradation products in total sample extracts (soil and water). During the study, tebupirimphos decreased from 93.5% at day 0 to 51.1% at day 127. The major degradates formed were *tert*-butylhydroxypyrimidine and desisopropyltebupirimphos. The highest concentration of desisopropyltebupirimphos was at day 127 at 19.4%. At day 127, *tert*-butylhydroxypyrimidine was present at 22.2% of the applied radioactivity. Minor products, the *S*-ethyl isomer of isopropyltebupirimphos and desethyltebupirimphos, were present in low amounts at less than 5% of the applied radioactivity. Initially, all of these degradates were tentatively identified by comparing HPLC retention times of the standards, and later, their structures were confirmed by mass spectrometry. Several minor products were also detected during the entire period of incubation; however, all of these were less than 1.0% of the applied radioactivity.

The identity of tebupirimphos and its degradation products, after isolating and purifying by TLC and/or HPLC, was confirmed by mass spectrometry using LC-MS-TSP and/or GC–MS. The degradates isolated from the samples were analyzed along with standards for comparison. Figures 1–3 show the mass spectra of degradates isolated from the samples. Figures 1 and 2 show the mass spectra of methyl derivatives of desisopropyltebupirimphos and *tert*-butylhydroxypyrimidine, respectively. Figure 1 shows the major peaks for desisopropyltebupirimphos at m/z 290 (parent ion), 275 (loss of methyl group), and 248 (loss of isocyanate from m/z 275). The major peaks in Figure 2 are m/z 166 (parent ion), 151 (loss of methyl group), and 124 (loss of isocyanate from m/z 151). These two products were also analyzed along with their standards by LC-MS-TSP. Thus, two separate techniques were used to confirm the structure of major degradates. The identity of two other products, desethyltebupirimphos and the *S*-ethyl isomer of isopropyltebupirimphos, was also confirmed by LC-MS-TSP in the positive-ion mode (Figure 3). In Figure 3A, the peak at m/z 305 represents the parent ion of the *S*-ethyl isomer of isopropyltebupir-

imphos, and in Figure 3B, the peak at m/z 291 represents the parent ion of desethyltebupirimphos.

A proposed degradation pathway for the metabolism of tebupirimphos under anaerobic aquatic conditions is shown in Figure 4. Desisopropyltebupirimphos was formed by direct hydrolysis in aqueous environment since this was the major hydrolytic product in the hydrolysis study using sterile buffer solutions at pH 5.0, 7.0, and 9.0 (Duah, 1990a). At these pHs, no difference in the rate of degradation of tebupirimphos was observed, and since the average pH in our system was 6.3, no adverse effect of pH was anticipated. Perhaps the formation of desethyltebupirimphos was also due to direct hydrolysis, although its amount was far less than desisopropyltebupirimphos. Both the *S*-ethyl isomer of isopropyltebupirimphos and *tert*-butylhydroxypyrimidine were the only metabolites formed in anaerobic soil metabolism study (Kao et al., 1990). In this study, *tert*-butylhydroxypyrimidine could have formed via several intermediates or by direct hydrolysis of tebupirimphos, as shown in Figure 4. The *S*-ethyl isomer of isopropyltebupirimphos was the result of rearrangement of the side chain.

Previous soil and aquatic studies on diazinon, which is a similar type of compound, have shown that the hydrolysis product, 2-isopropyl-6-methyl-4-hydroxypyrimidine, was the main metabolite (Sethunathan and MacRae, 1969; Sethunathan and Yoshida, 1969; Sethunathan and Pathak, 1972). Sethunathan compared its metabolism in both sterilized (streptomycin treated) and nonsterilized flooded soil and found that 2-isopropyl-6-methyl-4-hydroxypyrimidine is formed only under nonsterilized conditions. Thus, Sethunathan came to the conclusion that microbial hydrolysis of diazinon is more important than chemical hydrolysis (Sethunathan, 1973). In our study, desisopropyltebupirimphos and *tert*-butylhydroxypyrimidine were the main products. Desisopropyltebupirimphos was also the main hydrolytic product in the hydrolysis study using sterile, buffer solutions (Duah, 1990a) whereas *tert*-butylhydroxypyrimidine was one of the major metabolites in anaerobic soil metabolism (Kao et al., 1990). Thus, our results

Degradation of [14C]Tebupirimphos

J. Agric. Food Chem., Vol. 45, No. 6, 1997 **2353**

are in agreement with diazinon studies; the formation of *tert*-butylhydroxypyrimidine was probably due to microbial hydrolysis rather than chemical hydrolysis. Also, $^{14}CO_2$ formation was negligible from diazinon when applied to flooded soil (Sethunathan, 1973). Similarly, in our study, very few volatiles were formed. Thus, under anaerobic conditions, ring cleavage reactions did not appear to take place.

The half-life of tebupirimphos obtained under anaerobic aquatic conditions (194 days) is comparable to the half-lives calculated in other soil metabolism studies for the compound. The half-lives in aerobic and anaerobic soil metabolism studies were 343 and 279 days, respectively (Kao et al., 1990). An aqueous photolysis study gave the half-life of 31 h, indicating that tebupirimphos is a photolabile compound (Duah, 1990b). The half-lives obtained by field dissipation studies demonstrate the differences between laboratory and field studies. The field dissipation half-life of tebupirimphos in Minnesota soil was 73 days (Valadez, 1994; Dehart, 1995a), whereas the half-life in Illinois soil was 140 days (Dehart, 1994; 1995b). These differences clearly indicate that under natural conditions several factors may act on the compound simultaneously. Therefore, the half-lives obtained under laboratory conditions may vary significantly from those in nature.

In conclusion, we studied anaerobic aquatic degradation of [14C]tebupirimphos at a concentration of 2 ppm using sandy loam flooded with pond water. Under these experimental conditions, the half-life of tebupirimphos was calculated to be 194 days. The major products formed were *tert*-butylhydroxypyrimidine and desisopropyltebupirimphos, along with desethyltebupirimphos, the *S*-ethyl isomer of isopropyltebupirimphos, and several unidentified polar compounds as minor degradation products.

LITERATURE CITED

Dehart, B. A.; Mattern, G. C. Field dissipation of Phostebupirim (MAT 7484) on Illinois soil. Bayer Report No. 106240, 1994.

Dehart, B. A.; Lam, C. V.; Clay V. E. Recalculation of the field dissipation half-life of Phostebupirim (MAT 7484) in Minnesota soil. Bayer Report No. 106241-3, 1995a.

Dehart, B. A.; Lam, C. V.; Clay V. E. Recalculation of the field dissipation half-life of Phostebupirim (MAT 7484) in Illinois soil. Bayer Report No. 106240-3, 1995b.

Duah, F. K. Hydrolysis of MAT 7484 in sterile, buffer solutions. Bayer Report No. 99783, 1990a.

Duah, F. K. Photolysis of [pyrimidinyl-2-14C]MAT 7484 in an aqueous buffer solution. Bayer Report No. 100083, 1990b.

Kao, L. M.; Reichel, L. A.; Minn, A. Metabolism of [pyrimidinyl-2-14C]MAT 7484 in aerobic and anaerobic soil. Bayer Report No. 100323, 1990.

Sethunathan, N. Microbial degradation of insecticides in flooded soil and in anaerobic cultures. In *Residues of Pesticides and Other Contaminants in the Total Environment*; Gunther, F. A., Ed.; Springer-Verlag: New York, 1973; pp 143−164.

Sethunathan, N.; MacRae, I. C. Persistence and biodegradation of diazinon in submerged soils. *J. Agric. Food Chem.* **1969**, *17*, 221−225.

Sethunathan, N.; Yoshida, T. Fate of diazinon in soil-Accumulation of hydrolysis product. *J. Agric. Food Chem.* **1969**, *17*, 1192−1195.

Sethunathan, N.; Pathak, M. D. Increased biological hydrolysis of diazinon after repeated application in rice paddies. *J. Agric. Food Chem.* **1972**, *20*, 586−589.

Valadez, S. K.; Dehart, B. A. Terrestrial field dissipation of Phostebupirim (MAT 7484) on Minnesota soil. Bayer Report No. 106241, 1994.