APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1985, p. 737–743
0099-2240/85/040737-07$02.00/0

Vol. 49, No. 4

Cometabolism of Low Concentrations of Propachlor, Alachlor, and Cycloate in Sewage and Lake Water

NORMAN J. NOVICK AND MARTIN ALEXANDER*

Laboratory of Soil Microbiology, Department of Agronomy, Cornell University, Ithaca, New York 14853

Received 19 October 1984/Accepted 11 January 1985

Low concentrations of propachlor (2-chloro-*N*-isopropylacetanilide) and alachlor [2-chloro-2',6'-diethyl-*N*-(methoxymethyl)acetanilide] were not mineralized, cycloate (*S*-ethyl-*N*-ethylthiocyclohexanecarbamate) was slowly or not mineralized, and aniline and cyclohexylamine were readily mineralized in sewage and lake water. Propachlor, alachlor, and cycloate were extensively metabolized, but the products were organic. Little conversion of propachlor and alachlor was evident in sterilized sewage or lake water. The cometabolism of propachlor was essentially linear with time in lake water and was well fit by zero-order kinetics in short periods and by first-order kinetics in longer periods in sewage. The rate of cometabolism in sewage was directly proportional to propachlor concentration at levels from 63 pg/ml to more than 100 ng/ml. Glucose but not aniline increased the yield of products formed during propachlor cometabolism in sewage. No microorganism able to use propachlor as a sole source of carbon and energy was isolated, but bacteria isolated from sewage and lake water metabolized this chemical. During the metabolism of this herbicide by two of the bacteria, none of the carbon was assimilated. Our data indicate that cometabolism of these pesticides takes place at concentrations of synthetic compounds that commonly occur in natural waters.

Microorganisms that cometabolize substrates convert them to organic products but do not obtain energy from the reaction or use carbon for biosynthesis (1). Cometabolism by pure or mixed cultures of microorganisms has been extensively studied (7, 9, 13), and cometabolism in sewage (10), soil (3), and estuarine sediments (15) has also been investigated.

Although cometabolism may yield products which are toxic and persist in natural ecosystems, little information exists on the kinetics and factors affecting rates at chemical concentrations characteristic of those found in nature. Recent studies on compounds that are mineralized have shown the importance of using concentrations characteristic of those in nature because the rates observed at the high concentrations commonly tested in the laboratory cannot always be used to predict the rates at the low concentration characteristic of many bodies of natural water (14, 18). Moreover, a chemical may be mineralized at one concentration and cometabolized at another (22).

The aim of the present study was to characterize the metabolism of very low concentrations of three pesticides in sewage and eutrophic lake water. Factors affecting the rate of cometabolism of one of these compounds in samples from natural environments and in pure culture were also investigated.

MATERIALS AND METHODS

Chemicals. ^{14}C-labeled and unlabeled propachlor (2-chloro-*N*-isopropylacetanilide) and alachlor [2-chloro-2',6'-diethyl-*N*-(methoxymethyl)acetanilide] were provided by Monsanto Co., St. Louis, Mo., and cycloate (*S*-ethyl-*N*-ethylthiocyclohexanecarbamate) was supplied by Stauffer Chemical Co., San Francisco, Calif. The structures of these herbicides are shown in Fig. 1. Labeled aniline was supplied by Amersham Corp., Arlington Heights, Ill., and cyclohexylamine was supplied by CBN Corp., Sun Valley, Calif. Alachlor, propachlor, cycloate, and aniline were uniformly ring labeled and had specific activities of 7.1, 18.9, 41.6, and 1,100 μCi/mg, respectively. Cyclohexylamine was labeled on the C-1 carbon and had a specific activity of 11.1 μCi/mg. The levels of radiochemical purity of alachlor, propachlor, cycloate, aniline, and cyclohexylamine were 97, 97, 96, 99, and 98%, respectively, as determined by thin-layer chromatography.

Mineralization. The labeled compounds were added to 5 or 10 ml of primary effluent obtained from the Ithaca, N.Y., sewage treatment plant or to eutrophic lake water obtained from Beebe Lake, Ithaca, N.Y. The 100-ml serum bottles holding the samples were fitted with Teflon-coated silicon closures, from which 0.5-dram (1.85-ml) glass vials containing 1.0 ml of 0.33 N KOH were suspended. The triplicate samples and controls were incubated at 28°C on a rotary shaking operating at 100 rpm. To the sewage samples 2.0 ml of fresh or autoclaved sewage was added at weekly intervals. At the end of the test period, the samples were acidified by injecting 1.0-ml portions of 2 N H_2SO_4 through the caps, and the samples were incubated overnight on a shaker. The KOH solution was then placed in a scintillation vial with 16 ml of Liquiscint (Diagnostic Laboratories, Somerville, N.J.). The radioactivity in the samples was counted by using a model LS7500 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). The radioactivity in samples that had been autoclaved for 20 min was subtracted from the radioactivity in nonsterile samples.

Column chromatography. Sep-pak C_{18} cartridges (Water Associates, Milford, Mass.) were used to concentrate the various constituents and, in the case of propachlor and alachlor, to separate substrate from products. The C_{18} columns were premoistened by passing 2.0 ml of methanol and then 6.0 ml of water through them. When the columns were reused, 6.0 ml of methanol was added. The sewage or lake water sample was then passed through the column and, in the case of alachlor and propachlor, substrate and products were eluted in 1.0-ml fractions with combinations of hexane, ether, and methanol of increasing polarity. In other studies with propachlor, the products were separated from the substrate by eluting propachlor with two 4.0-ml portions

* Corresponding author.

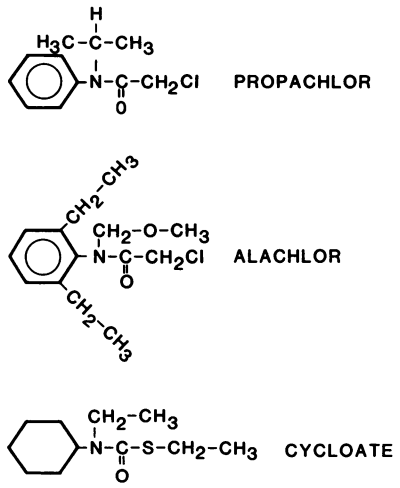

FIG. 1. Structures of the herbicides studied.

of anhydrous ether and the products with two 4.0-ml portions of methanol. To remove residual radioactivity from the column, 5.0 ml of 50% glacial acetic acid was used. Where appropriate, the fractions were collected in scintillation vials, and the radioactivity was counted by using 8 ml of Liquiscint. Alternatively, appropriate fractions were pooled, concentrated under N_2, and examined by thin-layer chromatography. Cycloate samples were concentrated on C_{18} columns, and the substrate and products were eluted with two 4.0-ml portions of anhydrous ethyl ether. The samples were then processed for thin-layer chromatography. The column removed more than 99% of the three labeled compounds from the aqueous sample at all concentrations tested.

Thin-layer chromatography. Samples of sewage or lake water were amended with 10 ng of labeled chemical per ml (either 100 ml of sample in 500-ml cotton-stoppered flasks for alachlor or propachlor or 10-ml portions in 100-ml serum bottles sealed with Teflon-coated silicon closures for cycloate). Autoclaved controls of all samples were run concurrently. Sewage samples were amended weekly with 20 ml (for alachlor and propachlor) or 2.0 ml (for cycloate) of fresh or autoclaved sewage.

After 6 weeks of incubation, the number and quantity of products resulting from the metabolism of propachlor and alachlor were determined. Two sterile and nonsterile samples of sewage and lake water amended with propachlor and alachlor were used to quantify product formation by separating product from residual substrate on a C_{18} column. Two additional sterile and nonsterile samples of sewage and lake water amended with the two pesticides were passed through C_{18} columns and processed for thin-layer chromatography in the following manner. Passing 1.0 ml of hexane through the column was found to remove water but not the labeled compounds, thus obviating the need for a later drying step. Methanol (8.0 ml) was then used to remove both products and residual propachlor. The methanol fraction was taken to dryness under a stream of N_2 at 35°C and suspended in 0.2 ml of methanol before spotting.

Sewage and lake water samples amended with cycloate were incubated for 8 and 14 weeks, respectively, and duplicate 10-ml portions from sterile and nonsterile samples from each environment were placed on separate C_{18} columns and eluted with ether as described above. Water was removed from the ether fractions by placing them over anhydrous Na_2SO_4, and the ether fractions were taken to dryness under a stream of N_2 at room temperature and then suspended in 0.2 ml of ether.

Portions of these solutions were spotted onto silica gel sheets (Eastman Kodak Co., Rochester, N.Y.). The fractions derived from environmental samples treated with propachlor and alachlor were run in a solvent system consisting of acetonitrile, water, and ammonium hydroxide (44:9:1), and the fractions from samples originally incubated with cycloate were separated by two-dimensional thin-layer chromatography in acetonitrile-water-ammonium hydroxide (44:9:1) in one direction and toluene-methanol-acetic acid (88:8:4) in the other. After the spots were located by autoradiography with Kodak X-omat AR film, the spots were cut from the sheets of gel, and radioactivity was determined by liquid scintillation counting.

Kinetics of propachlor metabolism. Sewage or lake water (50 ml) was added to cotton-stoppered 500-ml Erlenmeyer flasks. Control samples were autoclaved for 30 min or placed in 50-ml borosilicate glass tubes and exposed for 6 h to 2.5 Mrads of gamma irradiation. The irradiated samples were then transferred aseptically to sterile 500-ml flasks, 1.0 ng of labeled propachlor per ml was added, and the flasks were incubated at 28°C on a rotary shaker operating at 100 rpm. Individual autoclaved, irradiated, and nonsterile samples were removed periodically from the shaker, and the amount of propachlor converted to products was determined by separation on the C_{18} column.

To better characterize the kinetics of cometabolism, triplicate 150-ml portions of nonsterile or autoclaved samples in 500-ml flasks were amended with 10 ng of [^{14}C]propachlor per ml and incubated at 28°C on the shaker. At regular intervals, single 5.0-ml samples from each flask were placed on separate C_{18} columns. Substrate and products were separated as described above.

The relationship between substrate concentration and reaction rate was determined by amending duplicate samples of nonsterile and autoclaved sewage with 62 pg, 1.0 ng, 100 ng, 1.0 μg, 10 μg, and 100 μg of labeled propachlor per ml. The volumes of sewage used were 100, 50, and 5.0 ml for the 62-pg/ml, 1.0-ng/ml, and 10-ng/ml samples, respectively, and 2.0 ml for all other concentrations. The samples containing 62 pg/ml and 1.0 ng/ml were incubated in 500-ml cotton-stoppered flasks, and the other samples were incubated in cotton-stoppered glass scintillation vials. The samples containing 10 and 100 μg/ml contained 10% ^{14}C-labeled propachlor and 90% unlabeled analytical grade propachlor (99% pure), and all other samples contained only labeled propachlor. The flasks and vials were incubated for 45 h, and the substrate and products were separated on C_{18} columns.

To determine the effects of alternative substrates on the rate of propachlor metabolism, 5.0-ml portions of sewage in 100-ml cotton-stoppered serum bottles were either not amended or were amended with glucose or aniline and then treated with 10 ng of ^{14}C-labeled propachlor per ml. After 48 h, the quantity of products formed was determined.

Studies of individual species. Dilutions of sewage and lake water were plated onto a medium containing 0.05% yeast extract and 1.5% Bacto-agar (Difco Laboratories, Detroit, Mich.) in autoclaved sewage or Beebe Lake water. After 48 h, individual colonies were transferred to two sterile glass scintillation vials containing 2.0 ml of sterile sewage or lake water amended with 0.05% yeast extract. One of each duplicate set of vials received 100 ng of ^{14}C-labeled propachlor per ml. The vials were incubated for 48 h, and then those containing labeled propachlor were extracted

with 8.0 ml of ether. The ether layer was removed, and scintillation cocktail was added to the aqueous phase. Duplicates of vials showing increased radioactivity in the aqueous phase were streaked to assess the purity of the cultures, which were then tested again for activity.

Growth and propachlor metabolism by isolates BB16 and S13 were determined in duplicate 500-ml Erlenmeyer flasks containing 100 ml of a medium containing (per liter) 0.5 g of $(NH_4)_2SO_4$, 1.76 g of K_2HPO_4, 0.16 g of KH_2PO_4, 0.2 g of KCl, 0.2 g of $MgSO_4$, 0.1 g of NaCl, 50 mg of $CaCl_2 \cdot 2H_2O$, 20 mg of $FeCl_3 \cdot 6H_2O$, 0.2 g of glucose, and 10 μg of labeled or unlabeled propachlor. This medium was inoculated with 0.5-ml portions of a 36-h culture grown in the same medium but without propachlor. Cell numbers were determined by plating dilutions from flasks containing unlabeled propachlor onto half-strength Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.). Propachlor metabolism was determined by removing 5.0-ml portions from the flasks receiving the labeled chemical and separating substrates from products on a C_{18} column.

To determine whether the bacteria incorporated propachlor carbon, 10-ml portions of the glucose-salts solution supplemented with 10 ng of ^{14}C-labeled propachlor per ml were inoculated in duplicate with 0.1-ml portions of 36-h cultures. After incubation for 48 h, portions of the cultures were passed through 0.22-μm membrane filters (Millipore Corp., Bedford, Mass.), and the filtrates were collected in scintillation vials. The cells on the filters were washed three times with 5.0 ml of distilled deionized water. Each wash was collected in a separate scintillation vial, and 7.0 ml of scintillation cocktail was added to each vial. A portion of uninoculated medium was also filtered.

To determine the effects of alternative substrates on propachlor metabolism, 10-ml portions of the inorganic salts solution in cotton-stoppered 50-ml flasks were amended with 10 ng of [^{14}C]propachlor per ml and glucose, aniline, or phenol. The cultures were incubated for 4 days.

Isolation of bacteria metabolizing aniline or propachlor. Sewage (1.0 ml) was inoculated into 10 ml of inorganic salts solution supplemented with (per liter) 0.2 mg of $ZnSO_4 \cdot 7H_2O$, 25 μg of $CuSO_4 \cdot 5H_2O$, 0.2 mg of $MnCl_2 \cdot 4H_2O$, 10 μg of $Na_2MoO_4 \cdot 2H_2O$, 2 μg of $CoCl_2 \cdot 6H_2O$, 10 μg of H_3BO_3, and 50 mg of aniline or propachlor. After 1 to 2 weeks, 1.0 ml was transferred to 10 ml of fresh medium, and the new enrichment was incubated for 1 week. Aniline enrichments were plated onto the same medium supplemented with 0.02% aniline and 1.5% Bacto-Agar, and the colonies appearing in 48 h were restreaked twice.

TABLE 1. Mineralization of several chemicals in samples of sewage and lake water

Source of sample	Chemical	Concn (ng/ml)	Incubation time (weeks)	% Mineralized
Sewage	Propachlor	1, 10, 1,000	6	ND[a]
	Alachlor	10, 1,000	6	ND
	Cycloate	10	6	4.8
	Aniline	1	1	39.2
	Cyclohexylamine	10	1	63.3
Beebe Lake	Propachlor	1, 10, 1,000	6	ND
	Alachlor	10, 1,000	6	ND
	Cycloate	10	6	2.1
	Aniline	2	2	31.8
	Cyclohexylamine	10	4	53.0

[a] ND, Not detected.

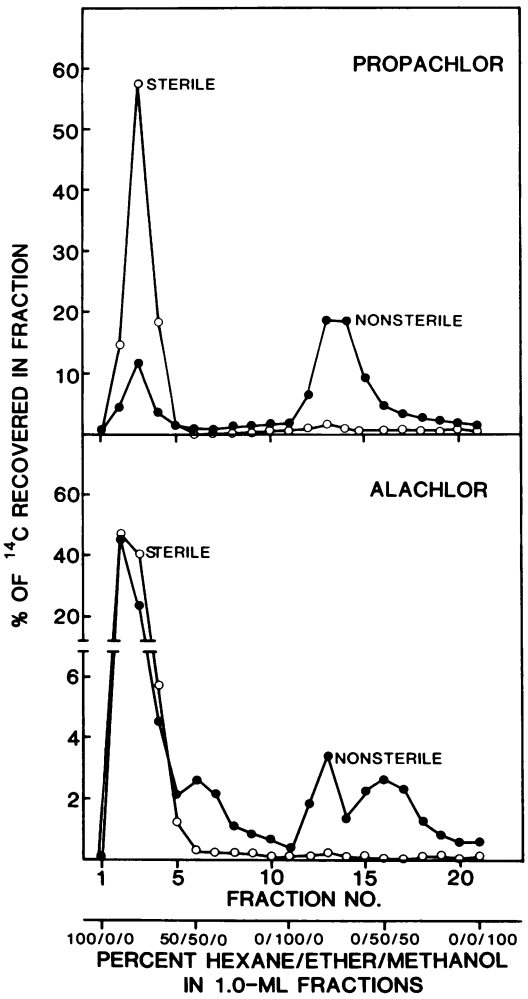

FIG. 2. Elution profiles from a C_{18} column of samples of sewage incubated with propachlor and alachlor.

RESULTS

Propachlor and alachlor were not mineralized in 42 days in sewage or Beebe Lake water at any concentration tested (Table 1). At a concentration of 10 ng/ml, 4.8 and 2.1% of the cycloate was mineralized in sewage and Beebe Lake water, respectively, in 42 days. Aniline and cyclohexylamine were readily mineralized in both sewage and lake water; one-third or more of these compounds was mineralized in the test periods.

The elution profiles from C_{18} columns of sewage samples incubated for 6 weeks with propachlor and alachlor are shown in Fig. 2. Mixtures (1.0 ml) of hexane and ether or ether and methanol in the ratios given at the bottom of Fig. 2 were passed through the column. The relative amounts of ether and hexane were serially increased or decreased in 0.1-ml increments in fractions 1 to 12, and the same was done for ether and methanol in fractions 12 to 21. Propachlor was eluted in fractions 2 to 4, and one or more metabolic products were eluted in fractions 12 to 16. Thin-layer chromatography indicated that only propachlor was present in fractions 2 to 4 and that this compound was absent from fractions 12 to 16. In all other experiments, propachlor was eluted with two 4.0-ml portions of ether, and the products were eluted with two 4.0-ml portions of methanol. Thin-

TABLE 2. Products resulting from the metabolism of herbicides in sewage and lake water

Chemical	Environmental sample	Incubation time (weeks)	% of substrate converted to products	No. of products
Propachlor	Sewage	6	81	12
Propachlor	Beebe Lake	6	59	14
Alachlor	Sewage	6	21	6
Alachlor	Beebe Lake	6	10	4
Cycloate	Sewage	8	37	3
Cycloate	Beebe Lake	14	51	5

layer chromatography indicated that the latter fractions contained no propachlor and that more than 90% of the ^{14}C in the former fractions was as propachlor. At least 80 to 85% of the radioactivity that disappeared from the ether fraction as a result of microbial metabolism was found in the methanol fraction, indicating the formation of polar products. The remaining radioactivity was retained on the column and could be eluted with 5.0 ml of 50% glacial acetic acid.

Alachlor was eluted from the column in fractions 2 to 5, and metabolic products formed from alachlor were found in several peaks between fractions 6 and 21. Thin-layer chromatography indicated that only alachlor was present in fractions 2 to 5; no alachlor was present in fractions 6 to 21. More than 95% of the radioactivity of alachlor lost during the 6-week incubation period from fractions 2 to 5 was recovered in fractions 6 to 21, indicating the formation of more polar compounds. The elution profiles of propachlor and alachlor incubated for 6 weeks with Beebe Lake water were essentially the same as those found in sewage.

Organic products were formed in sewage and lake water during the metabolism of 10 ng of propachlor per ml, 10 ng of alachlor per ml and 10 ng of cycloate per ml (Table 2). Although a large number of products were generated from propachlor, three accounted for 64 and 58% of the total product yield in sewage and lake water, respectively. During the metabolism of alachlor, the yield and number of products were lower than the yield and number of products for propachlor. Because much of the cycloate was lost by volatilization when it was added to sewage or lake water incubated in cotton-stoppered flasks, the tests were repeated with samples incubated in serum bottles sealed with Teflon-coated silicone closures. Under these conditions, 37 and 51% of the cycloate was converted to products in sewage and lake water, respectively. In addition, as much as 25% of the radioactivity from labeled cycloate added to sewage and lake water was lost during the incubation and processing of the samples. No such loss was evident from sterile sewage and lake water. The missing radioactivity was not recovered in the KOH trap and may represent a volatile product. The yield of products in sterile samples from the three chemicals was less than 15% of the yield found in the nonsterile samples.

The formation of products from the metabolism of propachlor in sewage and lake water is shown in Fig. 3. The conversion appeared to be nearly linear in lake water and sewage. More than 70% of the propachlor was converted to products in 21 days in Beebe Lake water, whereas 35% of the propachlor added to sewage was converted to products in 45 h. No products were found in autoclaved and gamma-irradiated sewage, but about 17% of the propachlor was converted to products in 30 days in water from Beebe Lake. The decrease in activity after 60 h in sewage and 21 days in lake water is not surprising because cometabolism requires a second organic substrate, the supply of which may be depleted in a long-term incubation, and because the microorganisms may lose activity with time in the absence of available nutrients. Nearly all of the propachlor could be converted to products if fresh sewage was added weekly to the flasks.

To better assess the kinetics of propachlor metabolism, triplicate flasks of nonsterile and autoclaved sewage were sampled at frequent intervals. The data were fit to the zero-order and first-order kinetics models with the zeta-correction term described by Simkins and Alexander (16) needed to analyze data from unfiltered samples. Both first-order and zero-order kinetics gave good approximations of the first eight sampling points (Fig. 4), with no significant difference between the approximations made with the two types of kinetics. First-order kinetics gave a better approximation when all 11 points were considered. The possible reasons for the decline in activity after 10 days are considered above.

The rate and extent of propachlor degradation varied in samples of sewage taken during this 18-month study. All samples metabolized propachlor, and the data were well fit by zero- and first-order kinetics, although the rate constants varied among samples.

The effect of concentration of propachlor on the rate of its metabolism in sewage is shown in Fig. 5. Because preliminary tests indicated that the rates were essentially linear for more than 45 h, the rates were determined by using the values obtained at 45 h. The rates were directly proportional to the substrate concentration at concentrations ranging from 63 pg/ml to more than 100 ng/ml. The rates were independent of substrate concentration at concentrations above 10 μg/ml. The V_{max} value was 5.5 ng/h per ml, and the K_m value was 2.1 μg/ml.

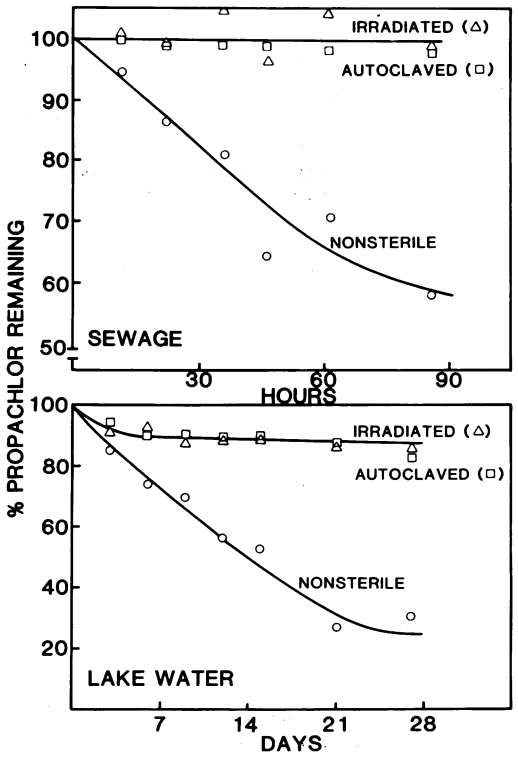

FIG. 3. Metabolism of 1.0 ng of propachlor per ml in sewage and Beebe Lake water.

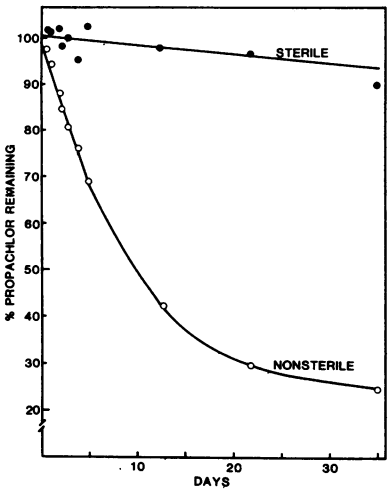

FIG. 4. Metabolism of propachlor added to sewage at a concentration of 10 ng/ml.

To determine the effect of other carbon sources on the metabolism of [^{14}C]propachlor (10 ng/ml), glucose or aniline was also added to the sewage. The addition of glucose doubled the yield of products generated from propachlor (Table 3). However, aniline at two concentrations had no effect on the activity.

Microorganisms capable of using propachlor as a sole carbon and energy source could not be isolated from sewage. Thus, turbidity did not appear in propachlor-enrichment cultures, and plating onto a medium containing 50 or 200 μg of propachlor per ml failed to show significant colony development. Nevertheless, bacteria able to grow on aniline were readily isolated by the same methods. Eight aniline-degrading isolates were inoculated into 50-ml flasks containing 8.0 ml of salts solution supplemented with trace elements and 200 μg of propachlor per ml, but none used propachlor (200 μg/ml) as a sole carbon and energy source. However, of 50 isolates obtained from sewage and 50 isolates obtained from lake water, 4 from sewage and 2 from lake water were found to cometabolize propachlor when they were incubated

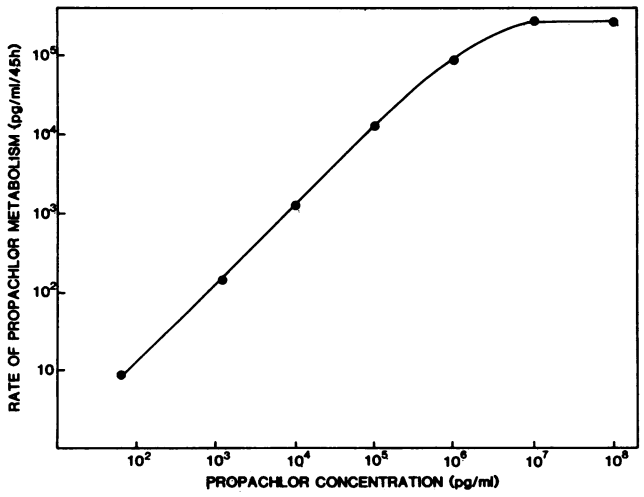

FIG. 5. Effect of propachlor concentration on the rate of propachlor metabolism in sewage.

TABLE 3. Effects of additional carbon sources on propachlor metabolism in sewage

Additional substrate	Concn (μg/ml)	% of propachlor converted to products
Glucose	200	62
Aniline	200	35
Aniline	10	32
None		32

for 7 days in a medium containing 0.05% yeast extract and 100 ng of propachlor per ml. The bacteria from sewage metabolized 71, 55, 29, and 28% of the propachlor, and those from lake water metabolized 92 and 27%. The isolates were different in their colonial and cell morphologies.

Because of their high activity, strains S13 and BB16 were used in further studies. Strain BB16 was a gram-negative rod-shaped organism with polar flagella. It was oxidase and catalase positive, did not ferment glucose, and probably was a strain of *Pseudomonas*. Isolate S13 was a gram-negative, nonmotile, rod-shaped organism which was oxidase and catalase negative and fermented glucose.

We conducted a study of the metabolism of propachlor by these two isolates when they were grown in a medium containing glucose. Both isolates grew readily in this medium, and they showed appreciable propachlor-metabolizing activity only late in growth (Fig. 6). When cells from the exponential and stationary phases of growth were harvested and suspended at equal densities, we found no differences in the ability to metabolize propachlor. Thus, the late appearance of product during the cycle of growth may have resulted from the time needed for the appearance of a sufficient number of cells to give detectable activity. From Fig. 6, we calculated that strains BB16 and S13 metabolized

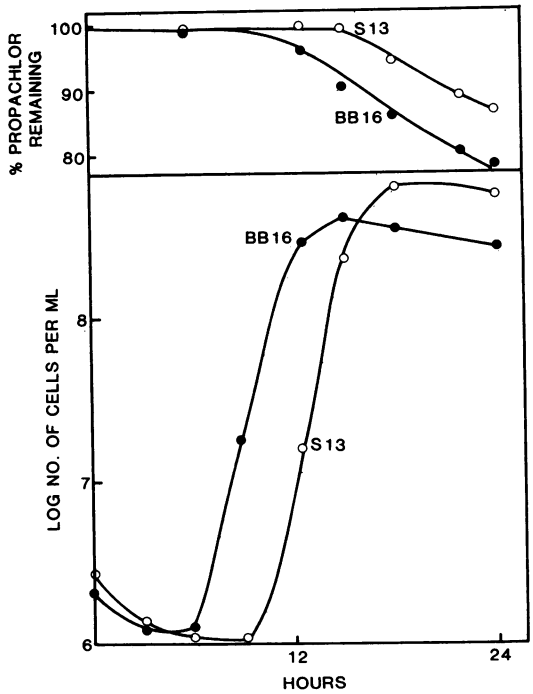

FIG. 6. Growth and propachlor metabolism by strains BB16 and S13 in an inorganic solution containing 200 μg of glucose per ml as the growth substrate.

TABLE 4. Effects of second carbon sources on the metabolism of propachlor by bacterial strain S13

Second carbon source	Concn (μg/ml)	% of propachlor converted to products
Glucose	10	13
Glucose	40	43
Glucose	200	90
Aniline	10	7
Aniline	200	11
Phenol	10	2
Phenol	200	5

9×10^{-10} and 5×10^{-10} ng of propachlor per h per cell, respectively.

The quantity of products resulting from the metabolism of 10 ng of propachlor per ml by strain S13 was related directly to the amount of glucose added as a carbon source (Table 4). The yield of products appeared to increase slightly at the higher levels of aniline and phenol tested. Carbon from propachlor did not seem to be incorporated into the bacterial cells. Thus, in experiments in which more than 50% of propachlor was converted to products by the two bacteria, the radioactivity retained on the 0.22-μm filters through which the cultures were passed was no greater (<7% of the radioactivity) than the radioactivity in uninoculated controls.

DISCUSSION

Under aerobic conditions microbial populations from sewage and eutrophic lake water are not able to mineralize the ring carbons of propachlor and alachlor in 6 weeks, and the mineralization of cycloate is at best very slow. Since cycloate was found to be 96% pure, the CO_2 may have originated not from the pesticide but from a contaminant. Organic products of all three compounds were found to accumulate in these environments, indicating that the molecules were cometabolized. Although microorganisms are chiefly responsible for the transformation of these compounds in soil (4, 23), alachlor and propachlor may be resistant to mineralization in soils, as well as in waste water and freshwater. Thus, Kaufman et al. (D. D. Kaufman, J. R. Plimmer, and J. Iwan, Proc. 162nd Natl. Meet. Am. Chem. Soc., Abstr. PEST-21, 1971) found that little $^{14}CO_2$ was evolved from soil treated with carbonyl-labeled propachlor or from pure cultures receiving this herbicide. Lee et al. (11) reported that *N*-isopropylaniline, *N*-isopropylacetanilide, *N*-(1-hydroxyisopropyl)-acetanilide, and *N*-isopropyl-2-acetoxyacetanilide were formed in soil treated with propachlor. Little of the ^{14}C from ring-labeled alachlor was converted to $^{14}CO_2$ in soil, but organic compounds were found to be generated from this pesticide by *Chaetomium globosum* (19). Although the present data show that cycloate is cometabolized in sewage and lake water and other investigators have shown that related compounds are cometabolized by pure cultures (7, 8, 20), this herbicide is mineralized in soil (23).

The rings of aniline and cyclohexylamine are readily cleaved in sewage and lake water, as indicated by the rapid mineralization of these compounds. Thus, structural characteristics other than the rings account for the resistance to mineralization of the three pesticides tested. It has been suggested that the presence of three substituents on the nitrogen atom in such compounds may account for their resistance (6).

Even under optimal conditions, the conversion of propachlor to products is extremely slow in sewage, lake water, and pure cultures. It is likely either that the enzymes catalyzing the conversions are not common in microorganisms or that the affinities of the enzymes for the synthetic compounds are much lower than the affinities for the natural substrates. No bacterium able to use propachlor as a sole carbon and energy source was isolated from sewage. Although the rate of cometabolism was proportional to substrate concentration, the extremely slow rate of transformation and the fact that populations do not maintain their activity for long periods preclude differentiation between zero- and first-order kinetics by the methods used.

The polar products which result from propachlor and alachlor degradation may have important environmental effects. Hydroxylation of the aliphatic portions of these compounds may lead to detoxication, as has been observed with the herbicides Karsil and chloranocyl (21), and the polar products may become tightly bound to sediments and soils, as has been shown with other pesticides (5). Such binding may affect the rates of biodegradation (17).

A compound such as glucose, which can support the growth of bacteria that cometabolize propachlor, can be used to increase the rate of propachlor metabolism in sewage and in pure culture. Glucose also has been reported to increase the rate of cometabolism of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) in sewage (12). Because the rate of cometabolism increased with increasing glucose concentration in pure culture, the enhancement by glucose may merely be a result of the presence of more cells able to metabolize propachlor.

Although propachlor and alachlor do not move readily through soil, low levels of both compounds have been detected in runoff water (2, 24, 25). The high volatility of cycloate makes it unlikely that high concentrations persist in natural water, but trace levels of this compound and perhaps less volatile products may persist. In this study we showed that mineralization of propachlor and alachlor did not occur in sewage or lake water but that organic products of the degradation of all three herbicides tested accumulated in samples from these environments.

ACKNOWLEDGMENTS

We thank S. Simkins for analysis of the kinetics.

This investigation was supported by funds provided by the U. S. Environmental Protection Agency under assistance agreement CR809735-02-0, by the U. S. Army Research Office, and by the U. S. Department of Agriculture under agreement USDA-TPSU-CU-2057-261.

LITERATURE CITED

1. **Alexander, M.** 1981. Biodegradation of chemicals of environmental concern. Science **211**:132–138.
2. **Baker, J. L., and J. M. Laflen.** 1979. Runoff losses of surface-applied herbicides as affected by wheel tracks and incorporation. J. Environ. Qual. **8**:602–607.
3. **Bartholomew, G. W., and M. Alexander.** 1979. Microbial metabolism of carbon monoxide in culture and in soil. Appl. Environ. Microbiol. **37**:932–937.
4. **Beestman, G. B., and J. M. Deming.** 1974. Dissipation of acetanilide herbicides from soils. Agron. J. **66**:308–311.
5. **Beynon, K. I., T. R. Roberts, and A. N. Wright.** 1974. Degradation of the herbicide benzoylprop-ethyl in soil. Pestic. Sci. **5**:451–463.
6. **Cripps, R. E., and T. R. Roberts.** 1978. Microbial degradation of herbicides, p. 669–730. *In* I. R. Hill and S. J. L. Wright (ed.), Pesticide microbiology. Academic Press, Inc., London.

7. **Davis, J. B., and R. L. Raymond.** 1961. Oxidation of alkyl-substituted cyclic hydrocarbons by a nocardia during growth on *n*-alkanes. Appl. Microbiol. **9**:383–388.
8. **de Klerk, H., and A. C. van der Linden.** 1974. Bacterial degradation of cyclohexane. Participation of a co-oxidation reaction. Antonie van Leeuwenhoek J. Microbiol. Serol. **40**: 7–15.
9. **Horvath, R. S.** 1971. Cometabolism of the herbicide 2,3,6-trichlorobenzoate. J. Agric. Food Chem. **19**:291–293.
10. **Jacobson, S. N., N. L. O'Mara, and M. Alexander.** 1980. Evidence for cometabolism in sewage. Appl. Environ. Microbiol. **40**:917–921.
11. **Lee, J. K., R. D. Minard, and J. M. Bollag.** 1982. Microbial metabolism of propachlor (2-chloro-*N*-isopropylacetanilide) in soil suspension. Han'guk Nonghwahakhoe Chi **25**:44–54.
12. **Pfaender, F. K., and M. Alexander.** 1973. Effect of nutrient additions on the apparent cometabolism of DDT. J. Agric. Food Chem. **21**:397–399.
13. **Rosenberg, A., and M. Alexander.** 1980. 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) decomposition in tropical soils and its cometabolism by bacteria in vitro. J. Agric. Food Chem. **28**: 705–709.
14. **Rubin, H. E., R. V. Subba-Rao, and M. Alexander.** 1982. Rates of mineralization of trace concentrations of aromatic compounds in lake water and sewage samples. Appl. Environ. Microbiol. **43**:1133–1138.
15. **Shiaris, M. P., and J. J. Cooney.** 1983. Replica plating method for estimating phenanthrene-utilizing and phenanthrene-cometabolizing microorganisms. Appl. Environ. Microbiol. **45**: 706–710.
16. **Simkins, S., and M. Alexander.** 1984. Models for mineralization kinetics with the variables of substrate concentration and population density. Appl. Environ. Microbiol. **47**:1299–1306.
17. **Subba-Rao, R. V., and M. Alexander.** 1982. Effect of sorption on mineralization of low concentrations of aromatic compounds in lake water samples. Appl. Environ. Microbiol. **44**:659–668.
18. **Subba-Rao, R. V., H. E. Rubin, and M. Alexander.** 1982. Kinetics and extent of mineralization of organic chemicals at trace levels in freshwater and sewage. Appl. Environ. Microbiol. **43**:1139–1150.
19. **Tiedje, J. M., and M. L. Hagedorn.** 1975. Degradation of alachlor by a soil fungus, *Chaetomium globosum*. J. Agric. Food Chem. **23**:77–81.
20. **van Ravenswaay Claasen, J. C., and A. C. van der Linden.** 1971. Substrate specificity of the paraffin hydroxylase of *Pseudomonas aeruginosa*. Antonie van Leeuwenhoek J. Microbiol. Serol. **37**:339–352.
21. **Wallnöfer, P. R., S. Safe, and O. Hutzinger.** 1973. Microbial hydroxylation of the herbicide *N*-(3,4-dichlorophenyl)-methacrylamide (dicryl). J. Agric. Food Chem. **21**:502–504.
22. **Wang, Y.-S., R. V. Subba-Rao, and M. Alexander.** 1984. Effect of substrate concentration and organic and inorganic compounds on the occurrence and rate of mineralization and cometabolism. Appl. Environ. Microbiol. **47**:1195–1200.
23. **Wilson, R. G.** 1984. Accelerated degradation of thiocarbamate herbicides in soil with prior thiocarbamate herbicide exposure. Weed Sci. **32**:264–268.
24. **Wu, T. L.** 1980. Dissipation of the herbicides atrazine and alachlor in a Maryland cornfield. J. Environ. Qual. **9**:459–465.
25. **Wu, T. L., D. L. Correll, and H. E. H. Remenapp.** 1983. Herbicide runoff from experimental watersheds. J. Environ. Qual. **12**:330–336.