Photochemical and Microbial Degradation of 2,4,5-Trichloroaniline in a Freshwater Lake

HUEY-MIN HWANG,^{1,2*} R. E. HODSON,¹ AND R. F. LEE²

Department of Microbiology, University of Georgia, Athens, Georgia 30602,¹ and Skidaway Institute of Oceanography, Savannah, Georgia 31416²

Received 22 February 1985/Accepted 19 August 1985

Surface waters from a eutrophic lake in northern Georgia were incubated with ¹⁴C-labeled 2,4,5-trichloroaniline to study the disappearance of the parent compound and production of ¹⁴CO₂. There was no degradation of the compound in the dark. Under 12 h of sunlight and 12 h of darkness, 28% of the trichloroaniline was degraded in both poisoned and untreated samples. Mineralization after 24 h in poisoned and untreated lake water was 5.5 and 6.8%, respectively. Thus, 81% of the mineralization was attributable to photochemical processes, and 19% was attributable to microbial processes. Most biological mineralization was due to microbes of bacterial size (<1.0 μm). Approximately 90% of the trichloroaniline bioaccumulated was associated with organisms larger than 1.0 μm, e.g., algae. When algae were removed by filtration, the amount of trichloroaniline mineralized increased to 9.4%, compared with 6.8% in the presence of algae. The excretion of organic compounds by algae may have inhibited bacterial mineralization of photoproducts.

A wide variety of organochlorine compounds are introduced into aquatic environments via domestic sewage, pesticide runoff from agricultural lands, and industrial effluents. The fate of the compounds depends on various biological, chemical, and physical processes. Most previous studies of the environmental fate of xenobiotics in aquatic environments have studied microbial or photochemical degradation separately (12, 18). Failure to study these two processes simultaneously can result in erroneous estimation of the degradation rate and can generate misleading information with respect to the persistence of the xenobiotics. For example, Pignatello et al. (18) found that photolysis accounted for 5 to 28% of the decline in pentachlorophenol. After acclimation, microbial degradation became the primary mechanism of pentachlorophenol removal in their flowing aquatic ecosystems. Radiolabeled dimethylbenzanthracene is not degraded in the dark but is mineralized in water samples exposed to sunlight because of microbial degradation of photooxidation products of the hydrocarbon (11). Photodegradation, however, does not necessarily render a parent compound harmless. For example, the photoproducts of aldrin and heptachlor are more toxic to the test animals than the parent compounds (9).

Direct photolysis of xenobiotics in aquatic environments is affected by the attenuation of sunlight by suspended particles and dissolved organics (14). In natural waters, photosensitized reactions can occur where humics and other chemicals initiate free radical oxidation of certain organic compounds (13, 19). Algae that contain photosensitizers, e.g., chlorophyll and flavoproteins, may promote photosensitized reactions. As autotrophs, algae may not metabolize xenobiotics, whereas bacteria, which have a greater variety of metabolic pathways, may be the major group of microbial degraders. Little information is available on the relative importance of algae and bacteria in the bioaccumulation or degradation of organic pollutants.

For this study we examined photochemical and microbial degradation of 2,4,5-trichloroaniline in the surface waters of Lago Lake, near Athens, Ga. The compound is a metabolite

of microbial degradation of various phenylurea and phenylcarbamate herbicides. With respect to degradation to CO₂, it is regarded as a recalcitrant pollutant in aquatic environments (22). This study represents an attempt at assessing the possible interactions between photochemical and microbial degradation of the compound. An incubation system was devised in which microbes and sunlight could simultaneously act on the compound. Photochemical and microbial degradation rates were determined. Size fractionation and specific inhibitor experiments were used to evaluate the relative contributions of autotrophic and heterotrophic microorganisms in degrading the pollutant.

MATERIALS AND METHODS

Sample collection. During July and August 1983, surface water samples were collected from Lago Lake, a small, shallow, moderately eutrophic lake near Athens, Ga. Water samples (4 liters each) were taken in acid-washed, 10-liter polyethylene containers. Assays were initiated within 1 h of collection. At the time of sampling, the temperature and pH were measured.

Incubation and degradation measurements. ¹⁴C-labeled 2,4,5-trichloroaniline (UL, 5.30 mCi/mmol; Pathfinder) was dissolved in acetone, and portions (20 μl; 0.1 μCi) were added to 40 ml of water in 160-ml Pyrex bottles (Corning Glass Works) that allow 90% transmission of light of wavelengths greater than 330 nm. The bottles were suspended in an outdoor tank, the temperature of which was maintained at 25°C. Water level in the bottles was 3 cm below the surface of the cooling water. Samples were incubated for various periods up to 1 day. Dark controls consisted of bottles covered with aluminum foil; abiotic controls contained mercuric chloride (1 mg liter⁻¹). All bottles were capped with silicon stoppers. At the termination of the incubation, 0.8 ml of 2 N H₂SO₄ was added to the sample. The ¹⁴CO₂ evolved was collected in a NaOH-saturated filter paper in a center well, which was attached to the stopper at time zero. The filter papers were then transferred to a second set of Pyrex bottles equipped with center well traps containing ethanolamine (Kodak), followed by the addition of 2 N H₂SO₄ to each bottle. After 4 h, the filter papers were transferred to

* Corresponding author.

TABLE 1. Degradation of 2,4,5-trichloroaniline by different fractions of lake water^a

Sample	Mineralization (¹⁴ CO ₂ produced)				Total degradation ^b			
	Abiotic (poisoned) ^c		Untreated		Abiotic (poisoned)		Untreated	
	July	August	July	August	July	August	July	August
Lake water	10.4 ± 0.2 (5.5 ± 0.1)	1.8 ± 0.2 (0.9 ± 0.1)	13.2 ± 0.2 ^d (6.8 ± 0.1)	5.2 ± 0.4 ^d (2.7 ± 0.2)	55.5 ± 2.1 (28.5 ± 1.1)	35.1 ± 1.4 (18.0 ± 0.7)	54.8 ± 2.7 (28.1 ± 1.4)	35.2 ± 1.0 (18.1 ± 0.5)
Lake water filtrate ^c								
3.0	9.0 ± 0.8 (4.6 ± 0.4)	2.7 ± 0.2 (1.4 ± 0.1)	15.1 ± 1.2 ^d (7.7 ± 0.6)	3.9 ± 0.2 ^d (2.0 ± 0.1)	37.2 ± 0.6 (19.1 ± 0.3)	33.7 ± 0.8 (17.3 ± 0.4)	53.3 ± 0.2 ^d (27.4 ± 0.1)	36.0 ± 0.4 ^d (18.5 ± 0.2)
1.0	9.3 ± 0.8 (4.8 ± 0.4)	3.3 ± 0.4 (1.7 ± 0.2)	18.4 ± 0.5 ^d (9.4 ± 0.3)	6.8 ± 0.4 ^d (3.5 ± 0.2)	47.6 ± 0.4 (24.4 ± 0.2)	39.9 ± 0.6 (20.5 ± 0.3)	60.4 ± 0.2 ^d (31.0 ± 0.1)	47.1 ± 0.4 ^d (24.2 ± 0.2)
0.6	11.3 ± 1.0 (5.8 ± 0.5)	2.1 ± 0.2 (1.1 ± 0.1)	18.1 ± 0.5 ^d (9.3 ± 0.3)	4.9 ± 0.2 ^d (2.5 ± 0.1)	50.8 ± 0.6 (26.1 ± 0.3)	36.6 ± 0.4 (18.8 ± 0.2)	70.2 ± 2.7 ^d (36.0 ± 1.4)	42.6 ± 0.8 ^d (21.9 ± 0.4)

^a The pH of the water was 7.4, and its temperature was 25°C. The trichloroaniline was added at a concentration of 78 µg liter⁻¹. The samples were incubated for 12 h in sunlight and 12 h in darkness. The incubation started at 12:00 p.m. and ended in 24 h in July and August. There was no degradation in the dark. Degradation is in 10³ cpm ± standard deviation (*n* = 3). The percent trichloroaniline degraded ± standard deviation (*n* = 3) is given in parentheses.

^b Refers to loss of parent compound, including mineralization.

^c Lake water was treated with mercuric chloride (1 mg liter⁻¹).

^d Significant difference from abiotic sample (*t* test; *P* ≤ 0.05).

^e Pore sizes are in micrometers.

scintillation vials containing Scintiverse I (Fisher Scientific Co.), and radioactivity was measured with a liquid scintillation counter (Beckman model LS-100C). The two-trap procedure was used to minimize counting interference due to adsorption of ¹⁴C-organic compounds onto the ethanolamine-soaked filter paper (4, 10). Trichloroaniline and its degradation products were recovered from 8-ml subsamples of the acidified water by three successive extractions with 1 ml of benzene. Recovery of the parent compound by this extraction procedure was greater than 90%. The extracts were applied to silica gel thin-layer chromatography plates (E. Merck AG) by using a solvent system of hexane-acetone (4:1, vol:vol). Trichloroaniline was detected by exposure to iodine. The parent compound and its degradation products were scraped from the thin-layer plates, and their radioactivity was determined.

Size fractionation of water samples. To determine the importance of different sizes of classes of microbes in degrading 2,4,5-trichloroaniline, we filtered water samples through various Nuclepore filters (Nuclepore Corp.; pore sizes, 3.0, 1.0, and 0.6 µm) before incubation with the compound. Dichlorophenyldimethylurea (Sigma Chemical Co.) was added to water samples by the procedures described by Vandermeulin et al. (20) to evaluate the magnitude of algal fixation of ¹⁴CO₂ derived from [¹⁴C]trichloroaniline. To study adsorption and bioaccumulation of [¹⁴C]trichloroaniline by particles, [¹⁴C]trichloroaniline was incubated with poisoned and untreated lake water under 12 h of sunlight and 12 h of darkness, followed by filtration first through 0.6-, 1.0-, or 3.0-µm-pore Nuclepore filters, and then on 0.2-µm-pore filters to collect particles. To test the possible inhibition on bacterial mineralization of the photoproducts of trichloroaniline by algal extracellular products, glycolate (free acid; Sigma) was added to 1.0-µm-filtered water, which then contained primarily bacteria.

Microbial biomass and activity measurements. Microbial heterotrophic activity was determined by adding ³H-labeled D-glucose (30 Ci/mmol; New England Nuclear Corp.) to unamended or size-fractionated subsamples of lake water. After incubating for 1 h, the samples were collected on

0.2-µm-pore Gelman filters, washed free of unassimilated label, and radioassayed by liquid scintillation spectrometry. Photosynthetic uptake of ¹⁴CO₂ was measured similarly by adding ¹⁴C-labeled sodium bicarbonate (50 mCi/mmol; New England Nuclear) to subsamples. After 3 h, the samples were filtered through 0.2-µm-pore Gelman filters, rinsed with filtered lake water, and radioassayed. Microbial biomass was estimated by assaying subsamples for total microbial ATP with the firefly luciferase assay of Holm-Hansen (6) after extraction with boiling bicarbonate buffer (5). Bacterial numbers in water samples were determined by direct microscopic counting with epifluorescence microscopy of acridine orange-stained specimens (3). Total chlorophyll (chlorophyll and pheophytins), used as a measure of phytoplankton biomass, was determined fluorometrically by procedures adapted from Holm-Hansen et al. (7).

RESULTS AND DISCUSSION

In July, water samples were incubated with trichloroaniline (78 µg liter⁻¹) under 12 h of sunlight and 12 h of darkness. The compound was not degraded in the dark. In both poisoned and untreated water samples incubated in the light, 28% of the compound was degraded (Table 1), suggesting that the initial degradation step was photochemical. These photoproducts could be further degraded to CO₂ by both photochemical and microbial processes, with 5.5 and 6.8% of the compound mineralized in poisoned and untreated water, respectively. Thus, 81% of the mineralization was attributable to photochemical processes, and 19% was attributable to microbial processes. Similar experiments were run in August with similar results, except that microbial processes accounted for a greater percentage (67%) of the total mineralization of the photoproducts (Table 1). The relative percentages of mineralization of [¹⁴C]trichloroaniline after 24 h in untreated water filtered through 3.0, 1.0, and 0.6 µm pores were 7.7, 9.4, and 9.3, respectively (Table 1). The 1.0- and 0.6-µm-pore filters excluded all of the carbon dioxide-fixing activity and most (>90%) of the chlorophyll (Table 2). The filtrate from 1.0-µm-pore filters re-

TABLE 2. Biomass and uptake of [³H]glucose or [¹⁴C]bicarbonate by different fractions of lake water^a

Sample	Acridine orange direct count (liter ⁻¹)	ATP (μg liter ⁻¹)	Total chlorophyll (μg liter ⁻¹)	[³ H]-glucose uptake ^b (%)	[¹⁴ C]-bicarbonate uptake ^c (%)
Lake water	2.2×10^{10}	4.2	24.5	27.2 (100)	1.7 (100)
Lake water filtrate ^d					
3.0	2.1×10^{10}	2.0	3.3	27.1 (100)	0.2 (10)
1.0	1.6×10^{10}	1.8	0.9	22.0 (81)	0 (0)
0.6	1.5×10^{10}	1.2	0.7	18.9 (69)	0 (0)

^a In July, the pH of the water was 7.4, and the temperature of the water was 25°C.

^b Water samples were incubated with 1.1×10^{-6} mM glucose for 1 h. Values are means of duplicate samples. The average difference in duplicate samples was less than 4%. The proportion of total uptake by each size fraction is given in parentheses. For example, in the 1.0-μm-pore filtrate, the proportion was $(22.0/27.2) \times 100\% = 81\%$.

^c Water samples were incubated with 8.0×10^{-4} mM bicarbonate in sunlight for 3 h.

^d Pore sizes are in micrometers.

tained 81% of the heterotrophic activity as indicated by uptake of [³H]glucose (Table 2). The higher trichloroaniline mineralization activity in water passed through 1.0- or 0.6-μm-pore filters relative to unfiltered water was presumably because of the absence of phytoplankton or protozoan bacterivores or both. Based on the fact that the ratio of bicarbonate turnover time in these water samples and incubation time in the degradation experiments was 15:1, we conclude that the reincorporation of ¹⁴CO₂ derived from trichloroaniline is negligible. Water samples treated with dichloro-phenyl-dimethylurea (0.1 mg liter⁻¹) an inhibitor of noncyclic photophosphorylation and electron transport in photosynthesis (8, 20), produced about 1.4 times as much ¹⁴CO₂ from trichloroaniline as from untreated water. Dichloro-phenyl-dimethylurea at this concentration blocked photosynthetic bicarbonate uptake in the samples but did not inhibit glucose uptake by bacteria. We speculate that extracellular products of algae could inhibit bacterial degradation of the photoproducts of trichloroaniline. In one experiment we observed significant inhibition (45%) of microbial mineralization of trichloroaniline photoproducts in samples to which had been added 10 μM glycolate, a known secretion product of many algae in aquatic environments (21).

We speculated that the absence of grazing on bacteria by protozoan bacterivores may have enhanced the bacterial mineralization rate in filtered waters. To test the effect of protozoan bacterivores, cycloheximide (0.1 mg liter⁻¹), an inhibitor of protein synthesis in eucaryotes (16, 17), was added to water samples. This compound did not affect mineralization of trichloroaniline.

Another possible explanation for the increased mineralization in filtered water is that filtration might increase the growth rate and metabolic activity of bacteria through the release of organic nutrients from algal cells by filtration. Data from this laboratory and work by Azam and Hodson (1, 2) indicated that the concentration of dissolved organic nutrients in filtered water samples remained constant when the filtration pressure differential was 25 cm Hg. Thus, the effect of filtration on bacterial metabolic activity was negligible in our experiments.

The increase in the photodegradation of trichloroaniline in water first passed through 0.6- or 1.0-μm-pore filters (Table 1) may be due to removal of algae that attenuate light and bioaccumulate trichloroaniline protecting against photodegradation (Table 3).

In poisoned and untreated water, the amount of [¹⁴C]trichloroaniline and its photoproducts on particles was 8.6 and 16.9%, respectively (Table 3). The adsorption of free

trichloroaniline (i.e., trichloroaniline not associated with particulates) to filters was negligible, so that 8.3% of [¹⁴C]trichloroaniline and its photoproducts was bioaccumulated. Results of the size fractionation studies indicated that particles larger than 1.0 μm accounted for most of the bioaccumulation (Table 3).

The extent of bioaccumulation of the trichloroaniline correlated with chlorophyll concentration ($r = +0.999$; Table 4), whereas biomineralization of trichloroaniline photoproducts negatively correlated with maximum particle size. Expressed on a per-unit ATP-biomass basis (Table 4), these trends are still the same. Therefore, algae were important bioaccumulators, whereas biomineralization of trichloroaniline photoproducts was carried out by smaller organisms, i.e., bacteria.

It is clear from this study that 2,4,5-trichloroaniline is not mineralized in the dark. After transformation of the compound by photolysis, the degradation products, presumably

TABLE 3. Adsorption and bioaccumulation of [¹⁴C]trichloroaniline and its photoproducts by particles in different fractions of lake water^a

Sample	10 ³ cpm ± SD (%) (n = 2)		
	Abiotic (poisoned) ^b	Untreated	Difference (bioaccumulation) ^c
Lake water	16.7 ± 0.4 (8.6 ± 0.2)	32.9 ± 1.6 (16.9 ± 0.8)	16.2 (8.3)
Lake water filtrate ^d			
3.0	10.7 ± 0.4 (5.5 ± 0.2)	13.4 ± 0.5 (6.9 ± 0.3)	2.7 (1.4)
1.0	8.8 ± 0.4 (4.5 ± 0.2)	10.6 ± 0.5 (5.4 ± 0.2)	1.8 (0.9)
0.6	7.4 ± 0.4 (3.8 ± 0.2)	8.4 ± 0.2 (4.3 ± 0.1)	1.0 (0.5)

^a Lake water samples were incubated for 24 h (12 h in sunlight and 12 h in darkness). The incubation started at 12:00 p.m. in July. The percentages of trichloroaniline and its photoproducts accumulated ± standard deviation (n = 2) are given in parentheses.

^b Water was treated with mercuric chloride (1 mg liter⁻¹).

^c Difference between poisoned and untreated water samples.

^d Water with [¹⁴C]-trichloroaniline and its photoproducts (78 μg liter⁻¹) was filtered through 0.6-, 1.0-, or 3.0-μm-pore Nuclepore filters as indicated after incubation, followed by filtration through a 0.2-μm-pore filter. The 0.2-μm-pore filter was counted.

TABLE 4. Correlation test of various parameters versus trichloroaniline mineralization and bioaccumulation^a

	Acridine orange direct count	ATP	Chlorophyll	Particle size ^b
Bioaccumulation	+0.732	+0.985*	+0.999**	+0.820
Biominingalization ^c	-0.756	-0.864	-0.929	-0.978*

^a Critical values for correlation coefficient are 0.950 at $\alpha = 0.05^*$ and 0.990 at $\alpha = 0.01^{**}$. Number of pairs of variates is 4; degree of freedom is 2.

^b Particles were assumed to be spherical, and the medians of the size ranges were taken as the means. For example, mean size of the 0.6- to 1.0- μm -pore fraction is 0.8 μm . However, 3.0 μm was set as the mean of the fraction greater than 3.0 μm .

^c Biominingalization is the $^{14}\text{CO}_2$ production corrected for abiotic $^{14}\text{CO}_2$ production in untreated water by different fractions as given in Table 2.

chloroaminophenol and catechols (15), were further degraded and finally mineralized by sunlight and microbes present in the lake water. Heterotrophic bacteria, $<1.0 \mu\text{m}$, were responsible for most of the biological mineralization. Microbial degradation accounted for 19 and 67% of the trichloroaniline mineralized in July and in August, respectively. Photolysis initiates transformation of trichloroaniline in water, with further degradation to CO_2 by microbes and sunlight. In short-term studies (less than 3 days), microbes could degrade only the photoproducts of trichloroaniline, but not trichloroaniline itself.

It is clear that microbial degradation kinetics and rates of other physical and chemical processes, such as photolysis and bioaccumulation and adsorption processes, must be assessed together to predict the persistence of organic compounds in aquatic environments.

ACKNOWLEDGMENTS

We thank Dannah McCauley for her typing service.

This work was supported by the Georgia Sea Grant Program, National Oceanic and Atmospheric Administration Office of Sea Grants, U.S. Department of Commerce, under grant NA80AA-D-00091.

LITERATURE CITED

1. Azam, F., and R. E. Hodson. 1977. Size distribution and activity of marine microheterotrophs. *Limnol. Oceanogr.* 22:492-501.
2. Azam, F., and R. E. Hodson. 1977. Dissolved ATP in the sea and its utilization by marine bacteria. *Nature (London)* 267:696-697.
3. Hobbie, J. E., R. J. Dailey, and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33:1225-1228.
4. Hodson, R. E., F. Azam, and R. F. Lee. 1977. Effects of four oils on natural marine bacterial populations: a controlled ecosystem pollution experiment. *Bull. Mar. Sci.* 27:119-126.
5. Hodson, R. E., A. E. Maccubbin, and L. R. Pomeroy. 1981. Dissolved adenosine triphosphate utilization by free-living and attached bacterioplankton. *Mar. Biol.* 64:43-51.
6. Holm-Hansen, O. 1973. Determination of total microbial biomass by measurement of adenosine triphosphate, p. 73-90. *In* R. R. Colwell and L. H. Stevenson (ed.), *Estuarine microbial ecology*. Belle W. Baruch Library of Marine Science, No. 1, University of South Carolina Press, Columbia.
7. Holm-Hansen, O., C. J. Lorenzen, R. W. Holmes, and J. P. H. Strickland. 1965. Fluorometric determination of chlorophyll. *J. Cons. Perm. Int. Explor. Mer.* 30:3-15.
8. Izawa, S., T. N. Connolly, G. D. Winget, and N. E. Good. 1966. Inhibition and uncoupling of photophosphorylation in chloroplasts. *In* Energy conservation by the photosynthetic apparatus. Brookhaven Symp. Biol. 19:169-187.
9. Khan, M. A. O., R. H. Stanton, D. J. Sutherland, J. D. Rosen, and N. Maitra. 1973. Toxicity-metabolism relationship of the photoisomers of certain chlorinated cyclodiene insecticide chemicals. *Arch. Environ. Contam. Toxicol.* 1:159-169.
10. Lee, R. F., and C. Ryan. 1979. Microbial degradation of organochlorine compounds in estuarine waters and sediments, p. 443-450. *In* A. W. Bourquin and P. H. Pritchard (ed.), *Proceedings of the workshop. Microbial degradation of pollutants in marine environments (EPA-600/9-79-1012)*. Environmental Protection Agency, Gulf Breeze, Fla.
11. Lee, R. F., and C. Ryan. 1983. Microbial and photochemical degradation of polycyclic aromatic hydrocarbons in estuarine waters and sediments. *Can. J. Fish. Aquat. Sci.* 40:86-94.
12. Matsumura, F. 1980. Use of a microcosm approach to assess pesticide biodegradability, p. 126-135. *In* A. W. Maki, K. L. Dickson, and J. Cairns (ed.), *Biotransformation and fate of chemicals in the aquatic environment*. American Society for Microbiology, Washington, D.C.
13. Mill, T., P. G. Hendry, and H. Richardson. 1980. Free-radical oxidants in natural waters. *Science* 207:886-887.
14. Miller, G. C., and R. G. Zepp. 1979. Effects of suspended sediments on photolysis rates of dissolved pollutants. *Water Res.* 13:453-459.
15. Miller, G. C., R. Zisook, and R. Zepp. 1980. Photolysis of 3,4-dichloroaniline in natural waters. *J. Agric. Food Chem.* 28:1053-1056.
16. Morris, I. 1966. The effect of cycloheximide (actidione) on protein and nucleic acid synthesis by *Chlorella*. *J. Exp. Bot.* 18:54-64.
17. Newell, S. Y., B. F. Sherr, E. B. Sherr, and R. D. Fallon. 1983. Bacterial response to presence of eukaryote inhibitors in water from a coastal marine environment. *Mar. Environ. Res.* 10:147-157.
18. Pignatello, J. J., M. M. Martinson, J. G. Steiert, R. E. Carlson, and R. L. Crawford. 1983. Biodegradation and photolysis of pentachlorophenol in artificial freshwater streams. *Appl. Environ. Microbiol.* 46:1024-1031.
19. Skurlatov, Y. I., R. G. Zepp, and G. L. Baughman. 1983. Photolysis rates of (2,4,5-trichlorophenoxy) acetic acid and 4-amino-3,5,6-trichloropicolinic acid in natural waters. *J. Agric. Food Chem.* 31:1065-1071.
20. Vandermeulen, J. H., N. D. Davis, and L. Muscatine. 1972. The effect of inhibitors of photosynthesis on zooxanthellae in corals and other marine invertebrates. *Mar. Biol.* 16:185-191.
21. Wright, R. T. 1975. Studies on glycolic acid metabolism by freshwater bacteria. *Limnol. Oceanogr.* 20:626-633.
22. Zeyer, J., and P. C. Kearney. 1982. Microbial metabolism of propanil and 3,4-dichloroaniline. *Pestic. Biochem. Physiol.* 17:224-231.