Microbial Degradation by Mineralization or Cometabolism Determined by Chemical Concentration and Environment

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Monuron [3-(4-chlorophenyl)-1,1-dimethylurea] was mineralized when added to sewage at a concentration of 10 μg/L but not at 10 mg/L. Organic products were formed at both concentrations. Products with the chromatographic characteristics of (4-chlorophenyl)urea and 4-chloroaniline were generated during the decomposition of the higher herbicide concentration. Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] and linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] were mineralized when added to sewage at a concentration of 500 ng/L but not at 2.0 mg/L. No evidence for cometabolism of the higher levels of these two herbicides was obtained, but significant amounts of an unknown product appeared at the lower diuron levels. Chlorobenzilate (ethyl 4,4′-dichlorobenzilate) was cometabolized in water samples from Beebe Lake and mineralized if the samples also contained freshwater sediments. Mineralization did not occur if glucose and inorganic nutrients were added to sediment-free lake water. Chlorobenzilate was transformed to organic products but not to CO_2 by microorganisms in water samples from three other lakes, but the pesticide was mineralized in sediment-containing water from two of those lakes. The results thus show that a pesticide may be cometabolized at one concentration or in samples from one type of environment and mineralized at a lower concentration or in samples from a different type of environment.

Recent evidence indicates that the occurrence of mineralization is a function of concentration. In some instances, a pesticide that is mineralized in natural waters at one concentration is not converted to CO_2 at lower levels (Boethling and Alexander, 1979). Conversely, a pesticide mineralized at low concentrations may not be converted to CO_2 at higher levels, even when the elevated concentration is below that usually deemed to be toxic to heterotrophic microorganisms (Rubin et al., 1982). Moreover, recent evidence indicates that isopropyl *N*-phenylcarbamate (IPC) may be mineralized at one concentration and apparently cometabolized, or at least converted in high yield to solely organic products, at another concentration (Wang et al., 1984). The susceptibility of an organic compound to either mineralization or cometabolism may also be determined by environmental factors.

The present study was designed to assess the possible influence of chemical concentration and type of environment on the susceptibility of several pesticides to mineralization and cometabolism. Because the effect of concentration on the occurrence and types of biodegradative processes has been studied with only a few chemicals, an investigation was initiated with phenylurea herbicides. These compounds were chosen because of their susceptibility to microbial metabolism in soils and waters (El-Dib and Aly, 1976; Hill et al., 1955; Sheets, 1964) and because they represent a class of important herbicides for which little is known about the influence of concentration. The chemical chosen to assess whether the type of environment may determine whether a chemical may be either mineralized or cometabolized was chlorobenzilate (ethyl 4,4′-dichlorobenzilate). This acaricide is of interest because its structural similarity to DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] suggests it may persist in natural ecosystems (Edwards, 1977; Johnson and Ball, 1972; Morley, 1977).

MATERIALS AND METHODS

The carbonyl-labeled diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], monuron [3-(4-chlorophenyl)-1,1-dimethylurea], and linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] had specific activities of 0.99, 0.41, and 1.7 mCi/mmol, respectively. Nonradioactive diuron, monuron, linuron, and other phenylureas with purities exceeding 99.9% were obtained from E. I. Du Pont de Nemours & Co., Wilmington, DE. Unlabeled chlorobenzilate (97% pure) and [ring-U-^{14}C]chlorobenzilate (specific activity, 13.7 mCi/mmol) were provided by Ciba-Geigy Corp., Greensboro, NC. No labeled contaminants were found by autoradiography of thin-layer chromatograms on silica gel of chlorobenzilate following separation with hexanes or acetonitrile–water–NH_4OH (81:17:2) as solvent systems.

Phenylurea Metabolism. Samples of sewage were taken from the settling tanks of the waste treatment plant in Ithaca, NY. The sewage samples were immediately passed through a glass fiber filter (no. 66085, Gelman Sciences, Inc., Ann Arbor, MI) to remove particulate matter, and either 5-L portions were incubated in 10-L glass bottles for the lower herbicide concentration or 0.6-L portions were incubated in 2-L Erlenmeyer flasks for the higher concentration. For the higher concentration, unlabeled diuron, monuron, or linuron were added to final concentrations of 2.0, 10, or 2.0 mg/L, respectively, and the labeled chemicals were added to give approximately 35, 35, or 60 dpm/mL, respectively. In the lower concentration, only the labeled chemicals were added to give 500 ng/L, 10 μg/L, and 500 ng/L, respectively. The glass containers were stoppered with foam plugs and incubated without shaking at about 20 °C. At regular intervals, 10- and 50-mL portions of sewage containing the higher and lower concentrations, respectively, were taken for radioactivity measurements, the 50-mL portions being concentrated 5-fold before assay. For thin-layer chromatography, 50- and 400-mL portions containing the higher and lower concentrations, respectively, were taken, concentrated to about 25 mL, and extracted three times with 25 mL of ethyl acetate; the extracts were combined, concentrated almost to dryness, and washed with a small volume of acetone.

Radioactivity was measured with a Beckman LS 7500 liquid scintillation spectrophotometer.

Sewage samples adjusted to 10 mL were acidified with concentrated H_2SO_4 to pH 2 and bubbled with air to remove $^{14}CO_2$. The treated samples and 10 mL of Redi-solve

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MP (Beckman Instruments, Fullerton, CA) were placed in 20-mL glass scintillation vials. The radioactivity of the samples was measured for 10 min. Details of the method are given elsewhere (Subba-Rao et al., 1982). Thin-layer chromatography of samples amended with phenylureas was carried out with precoated silica gel plates having a fluorescent indicator (Eastman Co., Rochester, NY) with chloroform–acetone (9:1) as developing solvent. Before placing samples on the plates, 1 drop of a 10% solution containing authentic chemicals in acetone was added to the samples. The separated authentic compounds were detected under UV light (259 nm). Portions of the gel were scraped individually, and the radioactivity in the gel was determined in scintillation vials with 7 mL of Aqueous Counting Scintillant (Amersham Corp., Arlington Heights, IL) as scintillator for radioactivity measurements.

For gas chromatographic analysis, 2.0, 10, or 2.0 mg of diuron, monuron, or linuron per L, respectively, was added to 1.2-L samples of filtered sewage contained in Erlenmeyer flasks. At regular intervals during the succeeding incubation period, 50-mL portions were taken and extracted three times with ethyl acetate. The extracts were combined, concentrated almost to dryness, and diluted to 0.5 mL with acetone. Portions (5 μL) were injected into the gas chromatograph. Gas chromatography was performed with a Perkin-Elmer gas chromatograph, Model 3920B, fitted with a flame ionization detector. The column was a 1.83 m × 3.18 mm stainless steel column packed with 3% silar 10C on 100/120 Gas Chrom Q (Applied Science Laboratories). The flow rate of the carrier gas, N_2, was about 45 mL/min. The temperatures of the interface and injection port were 260 and 240 °C, respectively. The column was maintained at 80 °C for 1 min, and the temperature was then increased to 240 °C at a rate of 16 °C/min.

Chlorobenzilate Metabolism. Samples of fresh water and oxic sediment were taken from Beebe and Cayuga Lakes (Ithaca, NY), Cayuta Lake (Odessa, NY), and Seneca Lake (Watkins Glen, NY). The samples of sediment were taken at a maximum depth of ca. 3 cm beneath less than 1 m of water. In studies of chlorobenzilate metabolism in fresh water receiving no nutrient addition, the nonradioactive and labeled acaricide in acetone were diluted with distilled water and added to 100-mL serum bottles containing 30 mL of lake water with or without 8.0 g of sediment (wet weight). The total amount of radioactivity added to each bottle ranged from 2.8 to 13.5 nCi. In investigations of chlorobenzilate transformation in freshwater amended with nutrients, 30 mL of lake water in 100-mL serum bottles was or was not supplemented with 0.2 g of sediment and 3.0 mg of glucose, 300 μg each of KH_2PO_4 and K_2HPO_4, and 180 μg of KNO_3 and incubated for 6 days. To each bottle was then added 5.4 nCi of labeled chlorobenzilate, which correspoonded to 4.3 μg/L.

Each combination of chlorobenzilate, sediment, or nutrients constituted a treatment, and each treatment was tested in duplicate. One third of each of the treatments received 500 mg of $HgCl_2$/L. A 10 × 75 mm test tube containing 2.0 mL of 0.4 N KOH was placed in each serum bottle, the top of the tube being above the level of water in the bottle. The bottles were sealed with teflon-faced, silicon septa and aluminum seals (Thomas Scientific, Philadelphia, PA) and incubated at 18 °C in the dark for a maximum of 22 days. Three serum bottles (two without and one with added Hg) from each treatment were periodically assayed for $^{14}CO_2$ collected in the KOH and for radioactivity in fractions eluted from liquid chromatography columns.

For liquid chromatography, 10 mL of lake water was removed from each serum bottle with a 10-mL plastic syringe fitted with a stainless steel needle, and the liquid was transferred to another 10-mL plastic syringe which was fitted with a liquid chromatography cartridge (SEP-PAK C_{18}, Waters Associates, Milford, MA). A 0.5-mL sample of lake water was removed for liquid scintillation counting. A plunger was inserted into the syringe, and the lake water was forced through the liquid chromatography cartridge. The effluent was weighed, and a 1.0-mL subsample was removed for liquid scinitillation counting. The cartridge retained all of the radioactivity in the portion of water assayed. Two 1.0-mL volumes of hexanes, diethyl ether, *tert*-butyl alcohol (maintained at 27 °C), and methanol in that sequence were passed through the cartridge, combined in plastic, 20-mL scintillation vials, and subjected to liquid scintillation counting. The combined radioactivity in hexane and diethyl ether fractions represented the amount of chlorobenzilate present, whereas the radioactivity eluted with *tert*-butyl alcohol and methanol represented the amount of organic products derived from chlorobenzilate. After a solvent was passed through the cartridge, it was flushed with 10 mL of air. The efficiency of eluting radioactivity from the cartridges was initially ca. 80% but diminished to ca. 65% after two weeks.

After removal of the lake water for liquid chromatography, 12 drops of concentrated H_2SO_4 was added to the serum bottles through the septa with a syringe and needle. The bottles were then shaken overnight, and the KOH in the test tubes was transferred into a 20-mL plastic scintillation vial, the tubes then being rinsed with 1.0 mL of distilled water. The rinse water and 8.0 mL of Liquiscint scintillation fluid (National Diagnostics, Somerville, NJ) were added to the scintillation vial. The vials were vigorously shaken and then allowed to remain stationary for 24 h prior to counting.

Radioactivity was measured with the liquid scintillation counter.

RESULTS

Phenylurea Metabolism. In sewage incubated with 2.0 mg of diuron or linuron or 10 mg of monuron per L for 100 days, no mineralization occurred as indicated by the constant amount of radioactivity in solution during this period. In contrast, mineralization was evident in sewage receiving 500 ng of diuron or linuron or 10 μg of monuron per L (Figure 1). No loss of radioactivity was noted at 30 days for linuron or 45 days for monuron, but all three chemicals were ultimately mineralized. At 84 days, 35.5, 39.2, and 19.9% of the ^{14}C from diuron, linuron, and monuron had disappeared from solution, and no further mineralization was observed at 100 days. Thus, the three herbicides were mineralized in sewage samples receiving the lower but not the higher concentrations.

Thin-layer chromatography was used to determine whether the phenylureas were converted to organic products even if they were not always transformed to CO_2. Under the test conditions, the R_f values of authentic diuron, monuron, and linuron were 0.73, 0.68, and 0.88, respectively. Of possible intermediates, tests with authentic compounds indicated that (3,4-dichlorophenyl)urea, (4-chlorophenyl)urea, and phenylurea would appear in the region with R_f values of 0.05–0.20, and 3-(3,4-dichlorophenyl)-1-methylurea, 3-(4-chlorophenyl)-1-methylurea, and 3-phenyl-1-methylurea would appear in the area with R_f values of 0.30–0.45.

In sewage incubated with 500 ng of diuron/L, the radioactivity in the TLC spot in which the herbicide would be found (R_f value from 0.80–0.65) declined slowly for the

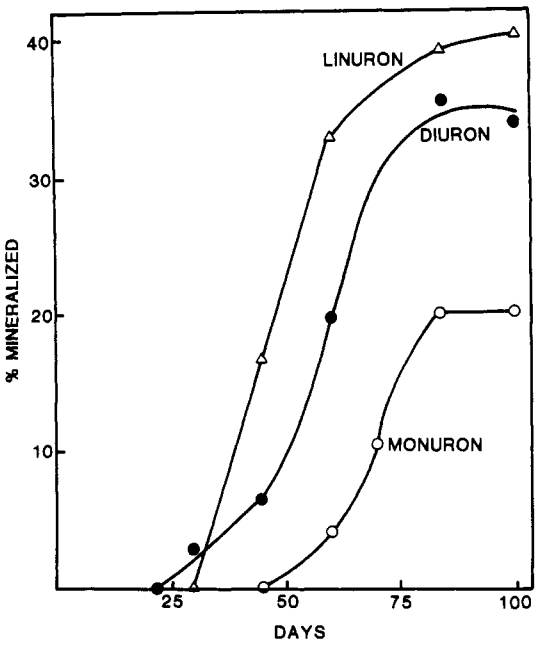

Figure 1. Mineralization of low concentrations of diuron, linuron, and monuron in samples of sewage.

first 15 days and then fell rapidly (Figure 2). At 60 days, only 6.0% of the initial radioactivity remained at that spot, although the total radioactivity remaining in the sewage was 80.2% at this time. An unknown product appeared in significant amount in the area with R_f value of 0.80–1.00, and the unknown accounted for 55% of the radioactivity at 60 days. A product appeared in the area having an R_f value of 0.30–0.45 and reached a maximum of 8.9% of the initial radioactivity, a yield that was evident at 45 days. In sewage receiving 2.0 mg of diuron/L, no radioactivity was lost from solution, and no appreciable or consistent changes in the amount of radioactivity occurred in regions having R_f values of 0.80–1.00.

In sewage amended with 10 μg or 10 mg of monuron/L, the radioactivity remaining in the spot containing the herbicide (R_f values of 0.60–0.75) declined markedly, and less than 10% of the ^{14}C was present in that region at 45 days (Figure 2). Nevertheless, no radioactivity was lost from the sewage at 45 days, and only 4.2% disappeared in sewage incubated for 60 days with 10 μg of monuron/L. As the herbicide, and possibly products cochromatographing with it, disappeared, one or more products appeared at regions of the TLC plates with an R_f value of 0.75–1.0. Small amounts of radioactivity appeared and sometimes disappeared with time of incubation at other regions of the chromatograms, but these changes never exceeded 5% of the initial radioactivity. By day 84, 19.9% of the radioactivity in solutions with 10 μg of monuron/L but none of that in solutions with 10 mg/L had disappeared from solution; i.e., mineralization, albeit slow, occurred at the lower but not the higher concentration.

In sewage receiving linuron at 2.0 mg/L, no loss of radioactivity was detected in a 60-day period, and no decline in radioactivity was evident in the spot in which this compound would be found (R_f value from 0.85–1.00). On the other hand, in sewage receiving 500 ng of linuron/L, a loss of radioactivity was evident at 45 days (Figure 2). At the same time, ^{14}C disappeared from the spot which contained the pesticide. No appreciable changes occurred during the 60-day period in the amount of ^{14}C recovered at any other portion of the TLC plates at either herbicide concentration.

Only 87–90% of the radioactivity of each herbicide was recovered at zero time at the spot on the chromatograms

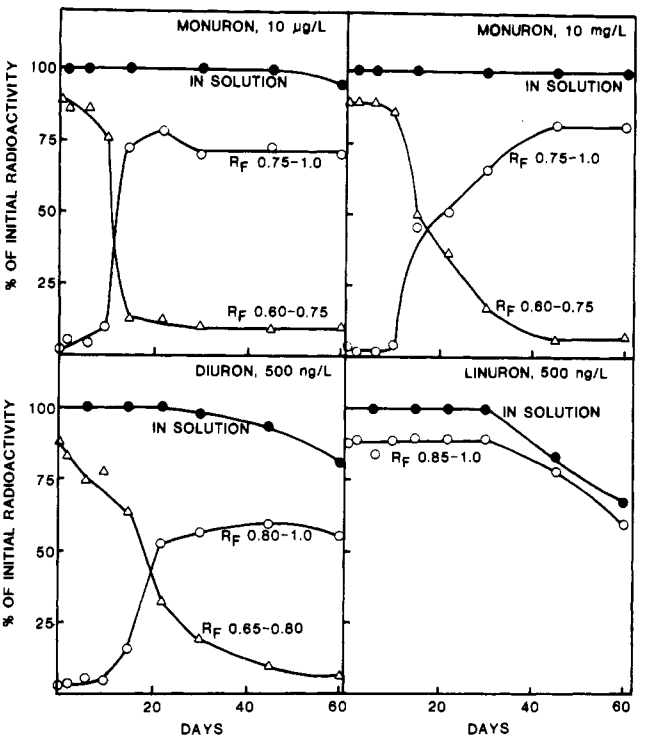

Figure 2. Mineralization of monuron, diuron, and linuron and changes in radioactivity at spots on thin-layer plates prepared from sewage receiving the three herbicides.

where the authentic compound should be found, although the chemicals were 99.9% pure. Because the recoveries at these spots exceed 99.9% with chemicals added to water rather than to sewage, it is likely that components of the sewage sorbed or reacted with the tagged pesticides.

Gas chromatography of authentic 4-chloroaniline and (4-chlorophenyl)urea showed distinct, sharp peaks with retention times of 7.6 and 3.8 min, respectively. For quantitative analysis, the peak height was compared with that of the standard compounds. In extracts of sewage incubated with 10 mg of monuron/L, in which product formation was detected, peaks corresponding to those of authentic (4-chlorophenyl)urea and 4-chloroaniline were found. The concentration increased to a maximum, which was equivalent to 0.25 mg of (4-chlorophenyl)urea and 0.19 mg of 4-chloroaniline/L at day 10, and then the level fell and reached values of less than 10% of the maximum by 60 days.

Algae developed in the sewage samples receiving the lower concentrations of the phenylureas because the sewage was incubated in the light in the laboratory. The algal contribution to mineralization was not evaluated, although algae may play a role in the degradation of phenylureas.

Chlorobenzilate Metabolism. In sediment-free Beebe Lake water amended with labeled chlorobenzilate, no $^{14}CO_2$ was produced. Nevertheless, the pesticide was transformed to organic products. This conversion was biological because chlorobenzilate did not disappear and products were not formed if the samples were amended with $HgCl_2$. The conversion of the acaricide to unidentified organic products was noted at concentrations of 3.2, 72, and 2,000 μg/L (Figure 3). The conversion during the period from 1 to 10 days was approximately linear, and the rate declined thereafter. Because the percentage conversion was approximately the same at the two lower concentrations and possibly slightly lower at the higher levels, the actual rate is roughly proportional to chemical concentration for the concentrations tested.

In contrast with the absence of mineralization in the

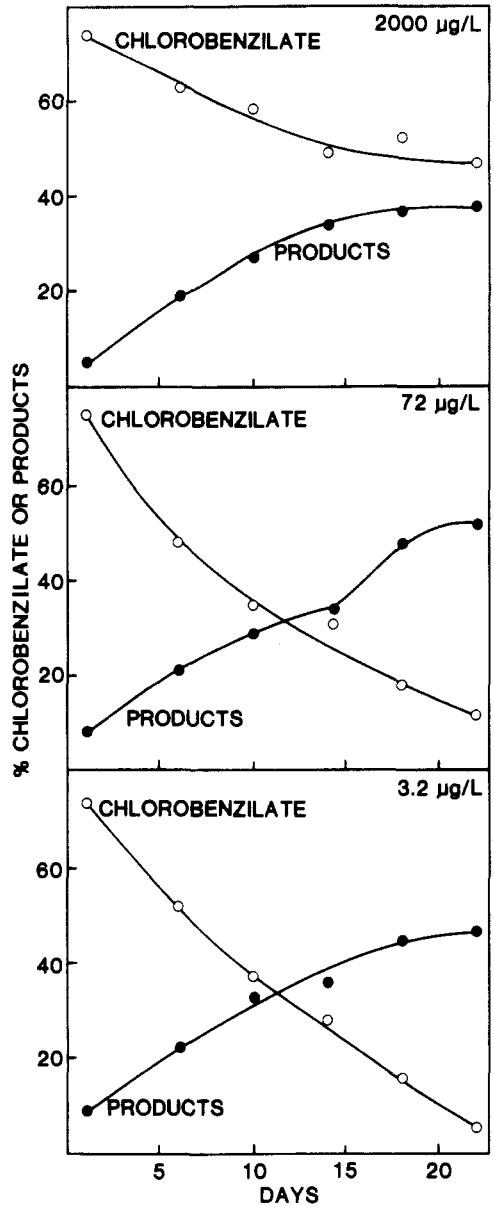

Figure 3. Cometabolism of chlorobenzilate at three concentrations in water samples from Beebe Lake.

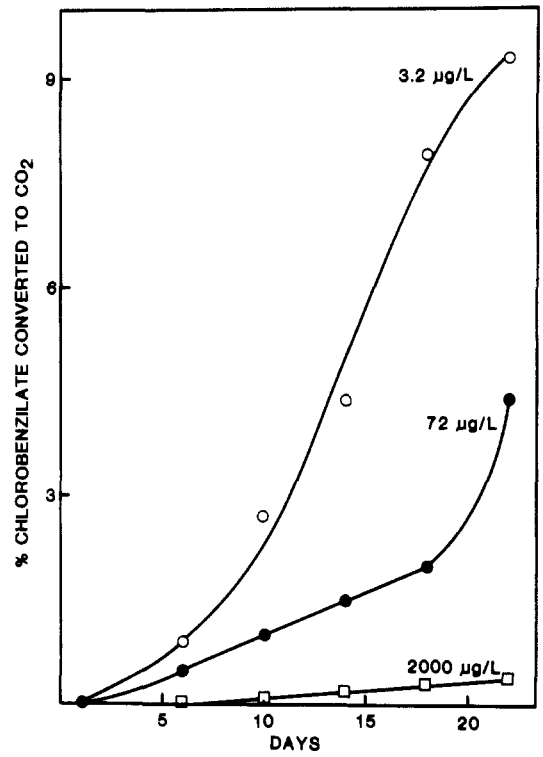

Figure 4. Mineralization of chlorobenzilate at three concentrations in mixtures of water and sediment from Beebe Lake.

samples of lake water, $^{14}CO_2$ was generated from labeled chlorobenzilate if the water contained sediment from Beebe Lake (Figure 4). Although the mineralization occurred at all three test concentrations of chlorobenzilate, the percentage conversion was lower as the concentration increased; hence, the absolute rate of mineralization was not directly proportional to concentration at the three levels tested. Because $^{14}CO_2$ was not produced if the samples received $HgCl_2$, the mineralization is biological.

In samples of sediment-free water from Cayuga, Cayuta, and Seneca Lakes, approximately 40, 29, and 39% of the chlorobenzilate added at 2.2 µg/L was converted to organic products in 22 days. During this time period, labeled CO_2 was not formed. However, if the lake waters contained sediment, 3.6, 0.0, and 18.3% of the tagged acaricide was converted to $^{14}CO_2$.

Samples of water from Beebe Lake were amended with 0.2 g of sediment from the same lake or allowed to remain sediment free. Half of the bottles received glucose and inorganic nutrients, and all the bottles were incubated for 6 days, by which time the nutrient-amended water was somewhat turbid and contained ca. 10^8 bacteria per mL. Each sample then received 4.3 µg of chlorobenzilate/L. After 16 days, no mineralization had occurred in sediment-free lake water receiving the nutrients but no sediment and in lake water amended with sediment but no nutrients; in contrast, 2.4% of the pesticide had been mineralized in nutrient-supplemented lake water that also contained sediment.

DISCUSSION

The data presented here show that conclusions on the microbial metabolism of pesticides at one concentration or in samples from one environment may not be valid relative to what may occur at another concentration or in another environment. The concentrations used in this study are not generally believed to be toxic to heterotrophic microorganisms, yet the microbial responses were markedly different. The findings are particularly relevent for testing for regulatory purposes because (a) the higher concentrations tested are those commonly used in routine assays of biodegradation, so that a conclusion based upon such a laboratory evaluation may give a false picture of what occurs at the lower levels in nature, and (b) the data suggest that a conclusion drawn from studies of samples from the water column of lakes or rivers may be invalid for chemicals that are sorbed to or otherwise come into contact with sediments.

The microbial conversion of an organic substrate under aerobic conditions to only organic products probably is a result of cometabolism. Cometabolism designates the metabolism by a microorganism of a substrate that the organism cannot use as a source of energy, carbon, or some other nutrient element. Presumably, communities of aerobic microorganisms using organic compounds as energy and/or carbon sources convert those substrates, at least in part, to CO_2. A compound thus is believed to be cometabolized if it is not converted to CO_2, organic products are formed, and the substrate is not converted to cellular products or is not a carbon source for microbial growth (Jacobson et al., 1980). Although the ability of microorganisms to assimilate carbon and to grow at the expense

of the pesticides was not tested, the absence of CO_2 formation from the chemical in aerobic conditions is good evidence for cometabolism. Moreover, the patterns of metabolism previously noted for monuron (Tillmanns et al., 1978) and chlorobenzilate (Miyazaki et al., 1969, 1970) are consistent with the view that the substrates are cometabolized.

The data show that monuron, diuron, and linuron were mineralized at the lower concentrations (10 or 0.5 μg/L) but not at the higher concentration. At the higher levels, monuron (10 mg/L), but not diuron or linuron (2.0 mg/L), was converted to organic products; these products possibly included (4-chlorophenyl)urea and 4-chloroaniline. It is not clear why monuron behaved differently from the other two phenylureas. Although it is not known why mineralization occurs at one concentration and cometabolism takes place at another or why mineralization occurs in waters containing sediment and cometabolism takes place in sediment-free waters, different organisms may be involved. In this regard, Rubin et al. (1982) suggested that phenol was mineralized at different concentrations by two different kinds of organisms, oligotrophs at the lower concentration and eutrophs at the higher concentration.

Cunninghamella echinulata was reported to convert monuron to (4-chlorophenyl)urea and 3-(4-chlorophenyl)-1-methylurea (Tillmanns et al., 1978). Kearney and Kaufman (1965) did not find 4-chloroaniline to be formed when monuron was used as the substrate for bacterial enzymes. Nevertheless, in the present studies, 4-chloroaniline and (4-chlorophenyl)urea appeared to be products generated from monuron in sewage, at least on the basis of cochromatography. A *Pseudomonas* sp. was found to mineralize 4-chloroaniline (Zeyer and Kearney, 1982). Little attention has been given to the metabolism of chlorobenzilate by microorganisms, although Miyazaki et al. (1969) reported the conversion of 4,4′-dichlorobenzilic acid to 4,4′-dichlorobenzophenone. The present findings indicate more extensive degradation inasmuch as CO_2 was generated from ring-labeled chlorobenzilate.

Registry No. Chlorobenzilate, 510-15-6; diuron, 330-54-1; monuron, 150-68-5; linuron, 330-55-2.

LITERATURE CITED

Boethling, R. S.; Alexander, M. *Appl. Environ.* Microbiol. **1979**, *37*, 1211.
Edwards, C. A. In "Pesticides in Aquatic Environments"; Khan, M. A. Q., Ed.; Plenum Press: New York, 1977; pp 11–38.
El-Dib, M. A.; Aly, O. A. *Water Res.* **1976**, *10*, 1055.
Hill, G. D.; McGahen, J. W.; Baker, H. M.; Finnerty, D. W.; Bingeman, C. W. *Agron. J.* 1955, *47*, 93.
Jacobson, S. N.; O'Mara, N. L.; Alexander, M. *Appl. Environ. Microbiol.* **1980**, *40*, 917.
Johnson, H. D.; Ball, R. C. In "Fate of Organic Pesticides in the Aquatic Environment"; American Chemical Society: Washington DC, 1972; pp 1–10.
Kearney, P. C.; Kaufman, D. D. *Science (Washington, D.C.)* **1965**, *147*, 740.
Miyazaki, S.; Boush, G. M.; Matsumura, F. *Appl. Microbiol.* **1969**, *18*, 972.
Miyazaki, S.; Boush, G. M.; Matsumura, F. *J. Agric. Food Chem.* **1970**, *18*, 87.
Morley, H. V. In "Pesticides in Aquatic Environments"; Khan, M. A. Q., Ed.; Plenum Press: New York, 1977; pp 53–74.
Rubin, H. E.; Subba-Rao, R. V.; Alexander, M. *Appl. Environ. Microbiol.* **1982**, *43*, 1133.
Sheets, T. J. *J. Agric. Food Chem.* **1964**, *12*, 30.
Subba-rao, R. V.; Rubin, H. E.; Alexander, M. *Appl. Environ. Microbiol.* **1982**, *43*, 1139.
Tillmanns, G. M.; Wallnoefer, R. R.; Engelhardt, G.; Olie, K.; Hutzinger, O. *Chemosphere* **1978**, *7*, 59.
Wang, Y. S.; Subba-Rao, R. V.; Alexander, M. *Appl. Environ. Microbiol.* **1984**, *47*, 1195.
Zeyer, J.; Kearney, P. C. *Pestic. Biochem. Physiol.* **1982**, *17*, 215.

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Diphenyl Ether Herbicides: Assignment of the Proton and Carbon-13 Nuclear Magnetic Resonance (NMR) Spectra of Acifluorfen, Acifluorfen Methyl, and Bifenox with Two-Dimensional NMR

G. H. Lee

A complete assignment of the proton and carbon-13 nuclear magnetic resonance (NMR) spectra of three diphenyl ether herbicides, i.e., acifluorfen [sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate], acifluorfen methyl [methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate], and bifenox [methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate], has been achieved based on data generated from coupling and two-dimensional NMR experiments.

INTRODUCTION

Derivatives of benzoic acid, e.g., dicamba and phenoxy acids, e.g., 2,4-D, have long been used as agronomically important herbicides. The search for a synthesized herbicidal activity between these two classes of chemicals has led to the discovery of several new diphenyl ether type herbicides, e.g., nitrofen and bifenox. Further refinement of the activity has led to the discovery of a new and very potent selective herbicide—acifluorfen (Johnson et al., 1978). Since the recent introduction of acifluorfen, numerous patents disclosing newer generations of this type of herbicide have appeared in the literature (Cartwright

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