Metabolic Fate of [^{14}C]Acrolein under Aerobic and Anaerobic Aquatic Conditions

Ann M. Smith* and John Mao

Environmental Science Division, Springborn Laboratories, Inc., 790 Main Street, Wareham, Massachusetts 02571

Rebecca A. Doane

Baker Performance Chemicals, Inc., 3920 Essex Lane, Houston, Texas 77227

Martin F. Kovacs, Jr.

Technical Assessment Systems, Inc., 1000 Potomac Street N.W., Washington, D.C. 20007

The metabolic fate of the irrigation canal herbicide MAGNACIDE H [active ingredient, acrolein (2-propenal)] was studied using natural sediment and water under aerobic and anaerobic aquatic controlled laboratory conditions. Test systems were treated at the recommended application rate of 15 mg/L. Water, sediment, and volatile trap analyses were performed to determine the chemical degradation pattern of acrolein. The half-life of acrolein was approximately 1 day, resulting in the production of several metabolic products that were ephemeral in nature. Identifications included 3-hydroxypropanal, acrylic acid, allyl alcohol, propionic acid, propanol, and 3-hydroxypropionic acid. Characterization and identification of metabolites were achieved using three different modes of HPLC separation: ion exclusion, reversed-phase, and anion-exchange chromatography. Bound residues were minimal (6–18% of applied dose). A comparison of degradation products and pathways, which ultimately lead to the production of oxalic acid and CO_2, under aerobic and anaerobic conditions is discussed.

Keywords: *Acrolein; 2-propenal; aquatic; metabolism; biodegradation*

INTRODUCTION

Acrolein, the active ingredient of an aquatic herbicide, MAGNACIDE H, is used to clear unwanted submerged and floating weeds and algae in irrigation ditches and canals, thereby maintaining the flow capacity of these channels. A highly reactive molecule with two reactive centers consisting of a C–C double bond and an aldehydic group (CH_2=CHCHO), acrolein is a general cell toxicant that reacts with the enzyme systems of these weed species and algae. It is also of great toxicological significance since it is an important synthetic intermediate in chemical industries (Beauchamp et al., 1985) and widespread human exposure to acrolein comes from tobacco smoke (Izard and Libermann, 1978). A complete understanding of acrolein degradation pathways under aerobic and anaerobic aquatic conditions is critical for ecological risk assessment of acrolein contamination in aquatic systems.

Acrolein is known to hydrolyze rapidly in water with first-order kinetic half-lives ranging from 15 to 60 h. The hydrolysis of acrolein in natural water was observed to proceed at a much faster rate than the degradation observed in buffered, distilled water (Bowmer and Higgins, 1976). Microbial transformation, therefore, was investigated as one of the competitive depletion mechanisms for acrolein. In this study, degradation of acrolein at the maximum application rate of 15 mg/L (15 ppm) in natural water and sediment aquatic conditions resulted in the production of the primary hydrolytic degradation product, 3-hydroxypropanal. Several metabolic products which were ephemeral in nature were also found, including acrylic acid, allyl alcohol, propionic acid, propanol, and 3-hydroxypropionic acid. Ultimately, oxalic acid and carbon dioxide (CO_2) were produced in this study.

To better follow the fate of acrolein in the irrigation canal sediment and water matrix, radiolabeled [2,3-^{14}C]-acrolein was used. [2,3-^{14}C]Acrolein was synthesized by Sigma Chemical Co., St. Louis, MO, to be used as a tracer of physical, chemical, and biological processes in aquatic metabolism studies. This investigation was the first to adequately demonstrate the degradation of acrolein into other small molecular weight, two- or three-carbon molecules and CO_2 through the use of three independent high-performance liquid chromatographs with radiometric detection (HPLC-RAM) methods (Mao et al., 1994).

The toxic effects of several diverse compounds in mammals have been related to their ability to form acrolein, an extremely reactive and toxic aldehyde (Beauchamp et al., 1985). The well-documented and diverse toxicities associated with acrolein have prompted investigation into the identification of enzymatic pathways responsible for detoxification of this aldehyde. The reaction of acrolein with molecules containing a sulfhydryl group has been reported by many authors (Draminski et al., 1983). Acrolein is a powerful electrophile that spontaneously reacts with sulfhydryl groups (Esterbauer et al., 1975) of vital enzyme systems. Conjugation with glutathione (Boor, 1985; Kaye, 1973) and oxidation of acrolein to acrylic acid (Patel et al., 1980; Rikans, 1987) have been observed to be enzymatic

* Author to whom correspondence should be addressed [fax (508) 295-8107].

0021-8561/95/1443-2497$09.00/0

pathways responsible for this detoxification. Some of these products, observed in mammals, were also measured under aerobic and anaerobic aquatic conditions. Mineralization to CO_2, also observed in this study, is supported by biodegradation experiments performed in other laboratories (Brink, 1976; Tabak et al., 1981; Kincannon et al., 1983).

MATERIALS AND METHODS

Chemicals. Radiolabeled [2,3-^{14}C]acrolein ([2,3-^{14}C]-2-propenal), lot 032H9223, 100 mCi, specific activity 16.4 mCi/mmol, was received from Sigma and stored under freezer conditions. The radiochemical purity was determined to be 92.2% by HPLC with radiometric detection. The chemical structure of [^{14}C]acrolein is C*H_2=C*HCHO (* indicating radiolabel positions). All nonradiolabeled reference standards were purchased from commercial sources and were of analytical grade. All solvents were of HPLC grade. The hydrolytic product, [^{14}C]-3-hydroxypropanal was synthesized by adding [^{14}C]-acrolein to water and allowing the mixture to react at room temperature for approximately 7 days. The 3-hydroxypropanal was used to determine HPLC retention time under different chromatographic conditions. The reaction mixture was allowed to react with pentafluorophenylhydrazine and analyzed by a particle beam liquid chromatography/mass spectroscopy system using electron impact ionization to confirm this reaction product.

Water and Sediment. The study was conducted using sediment and water collected from Kern County Canal, an irrigation canal located in a large agricultural region of California. The sediment was classified as a sandy loam that contained 0.5% organic matter. Before use, canal water and sediment were stored under refrigeration in the laboratory.

Experimental Setup. [^{14}C]Acrolein was added by gastight syringe to the water phase of each test system to obtain a dosing concentration of 15.0 mg/L, the maximum treatment rate allowed by label. The sediment/water ratio was 1:2. The test vessel, consisting of an all glass Erlenmeyer flask fitted with a glass Dreschel cap containing inlet and outlet ports for air or nitrogen exchange, was connected via silicone tubing to a series of volatile trapping vials or trapping tubes. Each test vessel was covered with aluminum foil to preclude exposure to light and incubated in an environmental chamber maintained at 25 ± 1 °C. For the anaerobic portion of this study, test systems containing untreated sediment and water were anaerobically incubated for approximately 1 month prior to treatment with [^{14}C]acrolein by daily 30-min purges with nitrogen. Headspace was removed from all flasks by daily air (aerobic) or nitrogen (anaerobic) purges throughout the study. To prevent the possibility of material loss through leaks, test systems were maintained under negative pressure. A vacuum pump was used to draw nitrogen from a high-purity cylinder to a 4-L nitrogen reservoir flask. An overflow flask, filled with water, maintained constant bubbling to ensure that the reservoir was completely filled with nitrogen. To minimize the possibility of external microbial contamination, the nitrogen was pulled through a sterile bacterial filter (PTFE Gelman Acro 50, 0.20 μm). Immediately prior to dosing, the outlet ports of each test vessel were connected to a series of trapping vials in the following order: one Tenax trap (manufactured by Supelco) and two 1.0 *N* NaOH traps (NaOH manufactured by Ricca Chemical Co.). The Tenax traps were used to collect volatile products in series with the two NaOH traps designed to collect $^{14}CO_2$. An empty trap was placed before and after each set of NaOH traps to preclude backflow contamination. The aerobic portion of the study continued for 32 days, and the anaerobic portion was 178 days in duration.

Sample Preparation *1. Water and Sediment.* The entire water/sediment sample was quantitatively transferred into a 200-mL centrifuge tube. The sample was centrifuged at 1000 rpm for 20 min and the water decanted. The final volume of the water fraction was measured, 3–4 mL was reserved for HPLC-RAM analysis, and triplicate aliquots were taken for radiometric liquid scintillation counting (LSC) analysis. All water samples were filtered through a 0.45 μm Acrodisc membrane filter prior to HPLC assay. After centrifugation, approximately 0.4 g of sediment was removed in triplicate for radiometric combustion analysis. An approximately 1-g sediment sample was taken for microbial biomass determination. Approximately 1.5 g of sediment was removed from duplicate tubes for percent moisture analysis. The percent moisture was determined by weighing the wet sediment samples and placing them in a fume hood to dry. The remaining sediment was extracted with 0.01 *N* NaOH. The extracts were analyzed by HPLC-RAM and LSC techniques. Sediment samples extracted with dilute NaOH solutions were neutralized to pH 7–8 with the addition of dilute HCl and then filtered through the 0.45 μm filter prior to HPLC-RAM analysis. After extraction, sediment samples were weighed and analyzed by radiometric combustion for bound, nonextractable residues. Additionally, some postextraction sediment was acidified to investigate the presence of bicarbonate in the sediment phase. Five grams of the sediment was placed in a Biometer flask, and 20 mL of 1 *N* NaOH was added to the side arm of the flask. The Biometer flask was attached to a trapping train consisting of one NaOH trap. Approximately 20 mL of 1 *N* HCl was added to the sediment, and the contents of the flask were stirred for 5 h. After stirring, the flask was left to stand for approximately 19 h. The supernatant was removed, and triplicate LSC analyses were taken of the supernatant, the NaOH from the side arm of the Biometric flask, and the NaOH from the trapping train. Postextraction combustion analyses were performed on the acidified sediment.

2. Trapping System. The NaOH trapping systems, removed and collected from each test vessel, were analyzed over the course of the study to preclude saturation. The total volume of the NaOH traps was measured and subsequently analyzed by LSC. The Tenax traps were eluted twice sequentially with 5 mL of methanol, and the eluent was analyzed by LSC. Representative NaOH traps were analyzed by a $Ba(OH)_2$ precipitation procedure to determine the presence of $^{14}CO_2$. In addition, canal water and aqueous sediment extracts were also analyzed by $Ba(OH)_2$ precipitation reaction to confirm the presence of $^{14}CO_2$. Samples were treated with a saturated $Ba(OH)_2$ solution (1:1 v/v), and the resulting precipitate was filtered. Precipitate and supernatant were subsequently analyzed by LSC.

3. Microbiology. The microbial biomass of sediment was determined at various time intervals throughout the duration of the study. An approximately 1-g sediment sample was collected from each test vessel for aerobic or anaerobic bacterial plate counts. Nutrient agar was used as the medium and was prepared according to the manufacturer's (Difco) instructions by dissolving the agar in sterile reagent grade water and sterilizing the final solution in an autoclave at 121 °C and 15 psi for 15 min. The sterilized medium was cooled to approximately 50 °C before use. The sediment sample was dissolved in 99 mL of sterile water and placed on a New Brunswick Scientific gyratory shaker table for 15–30 min. These samples were subjected to 1:10 serial dilutions using small sterile screw-cap test tubes containing 9 mL each of sterile water. An Eppendorf digital pipet with sterile tips was used to make transfers, and each test tube was vortexed before plating. Dilutions were carried out to the 10^{-6} range, resulting in a 10^{-7} dilution factor once a 100 μL aliquot had been plated. From each dilution, 100 μL aliquots were plated in triplicate using the previously prepared nutrient medium agar in the pour plate and swirl technique. After the agar solidified, the plates were placed in an incubator for aerobic samples or a BBL Gas Pack jar with an H_2 and CO_2 generating gas envelope, 10 mL of distilled water, a palladium catalyst, and an anaerobic indicator strip for anaerobic samples. The contents were fully sealed and placed in an incubator at approximately 25 °C for 48 h. The number of colony forming units per gram (cfu/g) of sediment was determined by counting for each plate.

4. Aerobicity and Anaerobicity. In addition to plate counts, aerobicity and anaerobicity were confirmed by periodic use of membrane electrodes placed directly in the aqueous phase of the test system. Aerobicity was determined by the use of a

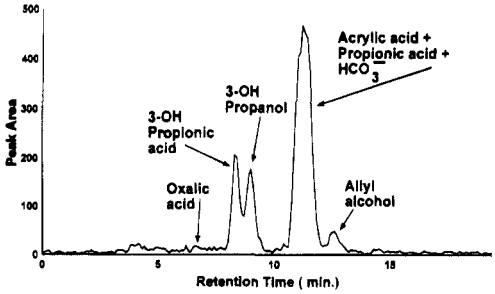

Figure 1. Ion-exclusion high-performance liquid chromatographic system provides separation of several acrolein degradation products in a day 2 aerobic water sample. The system utilized an Interaction ORH-801 organic acid column, 300 × 6.5 mm, at 35 °C, and 1 mM H_2SO_4 mobile phase at 0.8 mL/min. Acrylic acid, propionic acid, and bicarbonate coelute under these conditions.

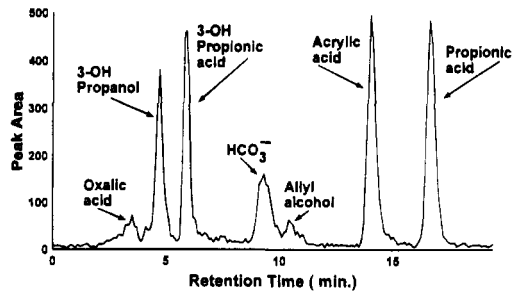

Figure 2. Reversed-phase high-performance liquid chromatographic system provides separation of several acrolein degradation products in a day 2 aerobic water sample. Three major metabolites, acrylic acid, propionic acid, and bicarbonate, were resolved under these conditions. The system utilized a Metachem Inertsil ODS-2 column, 5 μm, 250 × 4.6 mm, at 35 °C, and 0.05% H_3PO_4 mobile phase at 1 mL/min.

YSI dissolved oxygen probe and meter. Anaerobicity was measured by an Orion oxidation–reduction electrode and meter.

High-Performance Liquid Chromatography. The following instrumentation was used throughout the study for HPLC-RAM separation of acrolein and its degradation products: a Waters Model 510 solvent pump, a Hewlett-Packard Model 1050 autosampler, a Radiomatic Model A-280 radiometric detector with data acquisition systems, a FIAtron CH-30 column heater, a Hewlett-Packard Model 1047A refractive index detector, a Hewlett-Packard Model 1040A photo diode array detector, and a Hewlett-Packard Model 3396A integrator. The Radiomatic A-280 radiometric detector equipped with a 500-μL liquid scintillation cell was used to monitor radiolabeled components, while the refractive index and photo diode array detectors were used to determine retention times of nonradiolabeled reference standards. Three HPLC systems were used to fully characterize the many products that were formed during this study (Mao et al., 1994). Each water and sediment sample was analyzed using both the ion exclusion and reversed-phase chromatographic systems. Radioactive peaks were monitored using the flow-through Radiomatic A-280 radiometric detector. The scintillation cocktail (Radiomatic Flo-Scint A) was used at a flow rate of 2.4–3 mL/min for all analyses. The relative retention time of each radioactive peak to the parent acrolein peak was calculated for each sample. Peaks were assigned on the basis of their retention times relative to those of reference standards. To ensure that all radioactivities eluted off the column, a column recovery study was conducted with selected water samples. In this study, the HPLC eluent was collected at the outlet of the detector and assayed for radioactivity by LSC. The amount of collected radioactivity was compared to the amount of injected radioactivity, and the ratio of the two was defined as the column recovery. Column recoveries of greater than 90% were determined for all samples tested.

1. Ion Exclusion Chromatography. An ion exclusion HPLC chromatographic system used an Interaction ORH-801 organic acid column (300 × 6.5 mm) maintained at 35 °C and a 1 mM H_2SO_4 mobile phase at a fixed flow rate of 0.8 mL/min. The ion exclusion HPLC chromatographic system was used as the primary tool for peak assignment and quantification of each metabolite. The ORH-801 column was designed to separate low molecular weight polar organic acids and alcohols. Various analytical reference standards, including oxalic acid, malonic acid, glyceric acid, glyceraldehyde, lactic acid, glycerol, 3-hydroxypropanal, 1,3-propanediol, acrylic acid, and acrolein, were separated using this system. It is important to note that acrylic acid, propionic acid, and bicarbonate coelute under these conditions, as evidenced in a day 2 aerobic water sample presented in Figure 1. With the exception of acrylic acid, propionic acid, and bicarbonate, the quantification of each metabolite was done primarily using the ion exclusion system.

2. Reversed-Phase Chromatography. A reversed-phase HPLC chromatographic system used a MetaChem Inertsil ODS-2 column (5 μm, 250 × 4.6 mm) maintained at 35 °C and a 0.05% H_3PO_4 mobile phase at a fixed flow rate of 1 mL/min. The reversed-phase HPLC chromatographic system (system 2) was developed to resolve the three major metabolites (acrylic acid, propionic acid, and bicarbonate) shown in Figure 2 for the day 2 aerobic water sample. The reversed-phase chromatographic system was also used to confirm the peak assignment obtained using the ion-exclusion system. Separation of some analytical reference standards, including oxalic acid, glycerol, 1,3-propanediol, glycidol, malonic acid, allyl alcohol, acrylic acid, and aconitic acid, was obtained using the reversed-phase system.

3. Anion-Exchange Chromatography. An anion-exchange HPLC chromatographic system used a Phenomenex Spherex 10 SAX column (150 × 4.6 mm), maintained at ambient temperature, and a 5:95 acetonitrile/potassium hydrogen phthalate (2 mM, pH 6.5) mobile phase at 1.5 mL/min. Oxalic acid was found to be one of the primary degradation products in the sediment samples. Unfortunately, oxalic acid eluted first under both the ion exclusion and reversed-phase chromatographic systems (refer to Figures 1 and 2). To ensure identification with high confidence, an anion-exchange chromatographic system was developed to retain carboxylic acids (oxalic acid, malonic acid, etc.). Neutral compounds (alcohols) have little or no retention using this system. The existence of oxalic acid in sediment extract was, therefore, confirmed by the anion-exchange system.

Liquid Scintillation Counting. Liquid scintillation counting (LSC) analysis was used throughout the study to confirm recoveries from the HPLC-RAM columns and to affirm percent ^{14}C recovery vs ^{14}C applied in each phase of the test system. The total ^{14}C radioactivity in the water phase was determined by direct LSC. Aliquots of NaOH traps and eluent from Tenax traps were quantified by direct LSC. The sediment phase of each test system was analyzed by combustion and the total ^{14}C radioactivity measured by LSC techniques both prior to and after extraction. Sediment was oxidized in a Packard Model 306 Tri-Carb oxidizer and the $^{14}CO_2$ trapped in a mixture of Carbosorb and scintillation cocktail and counted utilizing a Beckman LS-1801 or LS-5000 liquid scintillation counter calibrated with factory-prepared standards.

RESULTS AND DISCUSSION

Microbial Populations. Acrolein is an effective biocide for certain water treatment applications (Brink, 1976). Despite the fact that acrolein has been used in a variety of antimicrobial applications (Hess et al., 1978), adequate colonies of aerobic and anaerobic microbial biomass were measured. Microbial populations in the natural environment range from as low as a few hundred cells per milliliter to as high as 10^9–10^{10} cells/mL (Brock, 1979). Aerobic microbial biomass at the conclusion of the 32-day study was approximately 5 × 10^7 cfu/g in sediment. The aerobic test system was also monitored by periodic use of a YSI dissolved oxygen

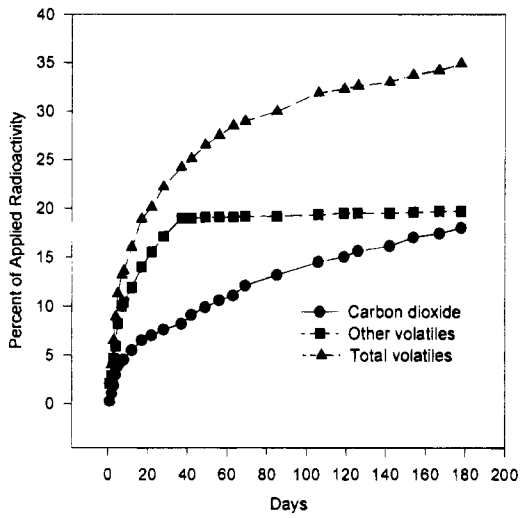

Figure 3. Cumulative trapping data collected during the anaerobic portion of the study. Results show up to day 42 more "other volatiles" were detected and thereafter more CO_2 was produced throughout the duration of the study. Data were obtained by treating NaOH traps containing CO_2 with $Ba(OH)_2$. The precipitate formed was comprised of barium carbonate and represented CO_2 concentration.

probe and meter to confirm aerobic conditions in the aqueous phase. Levels of 5–8 ppm of oxygen in the canal water were obtained. Anaerobic microbial biomass at the conclusion of the 178-day study was approximately 3×10^7 cfu/g in sediment. The anaerobic test system was monitored by periodic use of an Orion oxidation–reduction electrode and meter. Redox measurements in the range −200 to −300 mV were observed, confirming anaerobic conditions. The presence of adequate microbial colonies was attributed to the proper acclimation of this microbial system to the presence of acrolein at the concentration level tested. Acrolein has been found to be extremely toxic to microorganisms, but with properly acclimated cultures over 90% utilization of acrolein as substrate for the microbial community is possible (Chou et al., 1978).

Mineralization to CO_2. Mineralization of [^{14}C]-acrolein was observed, confirmed by the presence of $^{14}CO_2$ measured in all phases, i.e., water, sediment, and traps, of the test system. The primary degradation product in water was CO_2. Carbon dioxide represented 25% of the applied dose in the water and 6.4% in the sediment (total 3.7 ppm of CO_2 as acrolein equivalents) at termination of the aerobic study. Carbon dioxide represented 3.2% of the applied dose in the water and 3.8% in the sediment (total 0.7 ppm of CO_2 as acrolein equivalents) at termination of the anaerobic study. Oxalic acid and CO_2 were the predominant degradation products in the sediment for both the aerobic and anaerobic test systems at study termination. LSC analyses of the NaOH traps indicated that large percentages of the initial dose were volatiles. This radioactivity represented a combination of CO_2, acrolein, and alkali-soluble, volatile degradation products. The $Ba(OH)_2$ precipitation procedure performed with NaOH traps demonstrated that up to day 11 of the aerobic study and up to day 42 of the anaerobic study more volatiles other than CO_2 were produced. Thereafter and throughout the remainder of the study, more CO_2 was produced than other volatiles as depicted in Figure 3 for the anaerobic test systems. The mineralization of [^{14}C]acrolein to $^{14}CO_2$ observed in this study is supported by bench-scale activated sludge units which

Table 1. Rate Constants and Half-Lives of Acrolein under Aerobic and Anaerobic Aquatic Conditions in Natural Canal Water and Sediment

incubation type	test system phase	rate constant (k)	half-life $t_{1/2}$ (h^{-1})	correl coeff (r^2)	no. of observations (n)
aerobic	water	7.3×10^{-2}	9.5	0.554	16
aerobic	sediment	9.1×10^{-2}	7.6	0.673	16
anaerobic	water	6.7×10^{-2}	10.3	0.557	14
anaerobic	sediment	2.9×10^{-3}	239	0.673	20

established the biodegradation of acrolein (Brink, 1976). Biodegradability studies with toxic priority pollutants showed that biodegradation was the most important mechanism for the removal of acrolein (Tabak et al., 1981; Kincannon et al., 1983). Carbon dioxide was also observed as a degradation product of acrolein in mammals (Ghanayem et al., 1987).

Rate Constants and Half-Lives. The rate constants and half-lives of acrolein in natural sediment and canal water under aerobic and anaerobic aquatic metabolism conditions are presented in Table 1. Under the aerobic aquatic laboratory conditions employed in this study, acrolein was found to degrade rapidly in water and sediment with half-lives of less than 10 h. Under anaerobic aquatic laboratory conditions, acrolein degraded rapidly with a half-lives of approximately 10 h in the aqueous phase and approximately 10 days in the sediment phase.

Aerobic Aquatic Degradation of Acrolein. *1. Aerobic Degradation Pathway.* The degradation of acrolein through hydrolysis (Bowmer and Higgins, 1976) and mammalian metabolism (Patel et al., 1980; Draminski et al., 1983; Rikans, 1987) has been studied. The reactive nature of acrolein and the difficulties associated with identification of its small molecular weight, two- or three-carbon degradation products have led to an incomplete understanding of its degradation pathways. The complete characterization and identification of acrolein and its degradation products in this study was made possible by the three independent HPLC-RAM methods (Mao et al., 1994) used. All aerobic aquatic metabolites of acrolein determined in this study were polar, water-soluble, and less volatile than acrolein. Results of the aerobic portion of the study indicated that hydrolysis was one of the major degradation pathways, as evidenced by the formation of 3-hydroxypropanal. The biotransformation of acrolein under aerobic conditions was also demonstrated, as evidenced by the formation of acrylic acid and allyl alcohol. Oxidation of acrolein to acrylic acid by aldehyde dehydrogenase has been shown as an enzymatic pathway responsible for detoxification of acrolein in mammals (Patel et al., 1980; Rikans, 1987). Microbial transformation took place early in the aerobic study and competed with the hydrolysis process, as shown in Figure 4, which illustrates the early acrolein degradates formed. Acrolein then underwent self-oxidation and reduction to produce its oxidative product, acrylic acid, and its reductive product, allyl alcohol. The fate of allyl alcohol in the aqueous phase was not apparent, possibly due to its inherent volatility. Therefore, it was not present at the same levels as acrylic acid. Acrylic acid was reduced in the aqueous phase to propionic acid, which was then further oxidized to oxalic acid and eventually CO_2 through complete mineralization. 3-Hydroxypropanal was further oxidized in the aqueous phase to produce 3-hydroxypropionic acid. It has been postulated that 3-hydroxypropionic acid could be oxidized to malonic

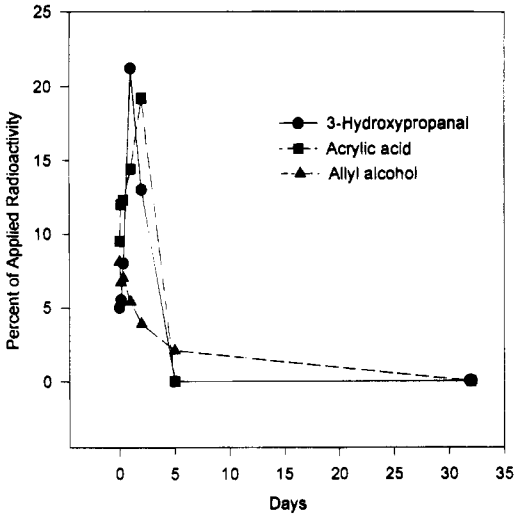

Figure 4. Percent of applied radioactivity measured as 3-hydroxypropanal, acrylic acid, and allyl alcohol by HPLC-RAM vs time for the aerobic portion of the study. These represent the early acrolein degradation products produced through day 2 of the 32-day study.

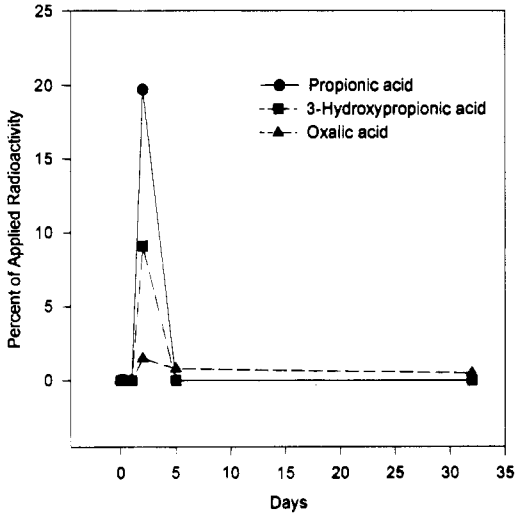

Figure 5. Percent of applied radioactivity measured as propionic acid, 3-hydroxypropionic acid, and oxalic acid by HPLC-RAM vs time for the aerobic portion of the study. These represent later acrolein degradation products produced on days 2–5 of the 32-day study.

acid and utilized in normal metabolic processes to form $^{14}CO_2$ (Ghanayem et al., 1987). The acrolein degradates produced after the initial hydrolytic and metabolic transformations are shown in Figure 5. An early acrolein degradation product, glyceric acid, was found only in the aerobic portion of the study. Acrylic acid was further oxidized to glyceric acid, which was then detected at 1.3% of applied radioactivity on day 5 of the aerobic portion of the study. The aerobic formation of glyceric acid may be indicative of the metabolism of an acrolein epoxide by epoxide hydrolase. Glyceric acid could also be further metabolized to glyceraldehyde, pyruvate, and ultimately $^{14}CO_2$ via entry into the tricarboxylic acid cycle (Ghanayem et al., 1987). Acrolein is hypothesized to act as the distal toxin responsible for the cellular damage caused by intoxication of several diverse compounds (Boor et al., 1987). These results indicated that allyl alcohol, allyl esters, and allylamine were metabolized *in vivo* to acrolein, which was conjugated with glutathione and ultimately excreted in urine as mercapturic acid (Boor, 1985; Kaye, 1973). The formation in this study of the acrolein

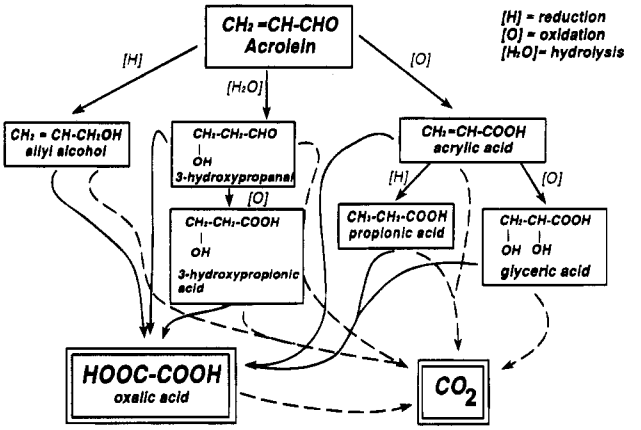

Figure 6. Production of oxalic acid and CO_2 for acrolein metabolism under aerobic aquatic conditions comprised oxidative, reductive, and hydrolytic reactions. Some of the intermediary steps remain unknown. 3-Hydroxypropionic acid could be oxidized through malonic acid to CO_2. Glyceric acid could be indicative of the metabolism of an acrolein epoxide by epoxide hydrolase.

degradation products allyl alcohol, acrylic acid, propionic acid, and 3-hydroxypropionic acid indicated that the enzymatic reactions observed in mammals could also occur in microbial systems. A proposed metabolic pathway for acrolein under aerobic aquatic conditions is illustrated in Figure 6. The review of some aquatic toxicity literature and the degradation of acrolein in water suggest that toxicity concerns to aquatic organisms come from acrolein and not its metabolites. There are no aquatic toxicity data for 3-hydroxypropanal, the hydrolytic product of acrolein. 3-Hydroxypropanal is a difficult compound to synthesize, which may be one reason for the lack of aquatic toxicity data. Acrylic acid also has limited available toxicity information, but as a three-carbon organic acid, it is not likely that it would be more toxic than acrolein. Bicarbonate would not be toxic to aquatic organisms in relation to its production from an acrolein application. Allyl alcohol and oxalic acid are considerably less toxic to fish than acrolein. Glyceric acid, another three-carbon organic acid, was found in only one of three samples.

2. Distribution of Radioactivity. At the termination of the 32-day aerobic study, approximately 25% of the radioactivity dosed to the test system was detected in the aqueous phase, while the radioactivity in the sediment phase corresponded to approximately 20% of the applied dose. The decrease in radioactivity observed in the aqueous phase was attributed to the rapid mineralization of acrolein degradates to $^{14}CO_2$. Consequently, $^{14}CO_2$, which was the major product in the volatile trap, constituted approximately 40% of applied dose on day 32. The mineralization of acrolein degradates also took place in the sediment phase, which was evidenced by the formation of bicarbonate anions, which were strongly adsorbed to the sediment and accounted for at least half of the sediment extractable radioactivity (i.e., 7% of the applied dose). Bound residues, i.e., radioactivity that was not removed from the sediment after extraction with dilute NaOH, also constituted approximately 7% of the applied radioactive dose at the termination of the 32-day aerobic study.

Anaerobic Aquatic Degradation of Acrolein. *1. Anaerobic Degradation Pathway.* The overall degradation of acrolein was not only microbially mediated through biodegradation but also coupled with hydrolytic chemical degradation. The hydrolytic degradation path-

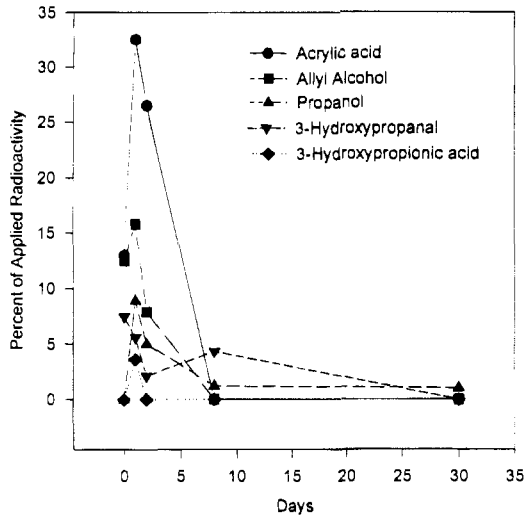

Figure 7. Percent of applied radioactivity measured as acrylic acid, allyl alcohol, propanol, 3-hydroxypropanal, and 3-hydroxypropionic acid by HPLC-RAM vs time for the anaerobic portion of the study. These represent acrolein degradation products produced through day 3 of the 178-day study.

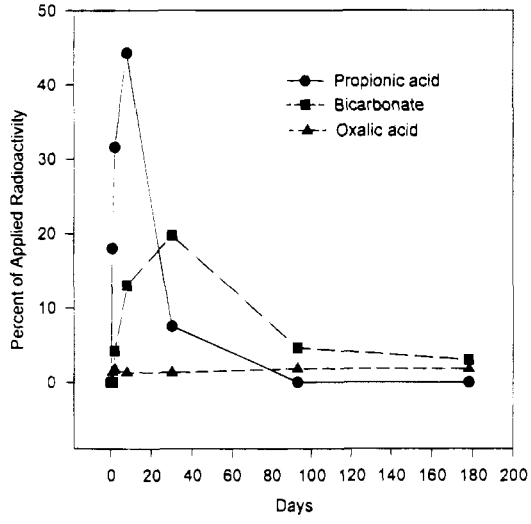

Figure 8. Percent of applied radioactivity measured as bicarbonate, oxalic acid, and propionic acid by HPLC-RAM vs time for the anaerobic portion of the study. These represent the later acrolein degradation products produced after day 3 of the 178-day study.

way of acrolein under both aerobic and anaerobic aquatic conditions was confirmed by the formation of 3-hydroxypropanal (Bowmer and Higgins, 1976). 3-Hydroxypropanal was then further oxidized in the aqueous phase to produce 3-hydroxypropionic acid. The oxidative product of acrolein, acrylic acid, and the reductive product, allyl alcohol, were produced in the aqueous phase during the early stages of the anaerobic study. Allyl alcohol was further reduced to propanol, an early acrolein degradation product found only in the anaerobic study. In the aqueous phase, the acrylic acid formed was further reduced to propionic acid, which on day 8 of the anaerobic study constituted greater than 40% of the applied radioactive dose. All of these transient degradation products were then further transformed in the aqueous phase to the terminal degradation products, oxalic acid and CO_2. Plots of both the earlier and later anaerobic degradation products of acrolein produced in the aqueous phase are presented in Figures 7 and 8, respectively. A proposed metabolic pathway for acrolein under anaerobic aquatic conditions is illustrated in

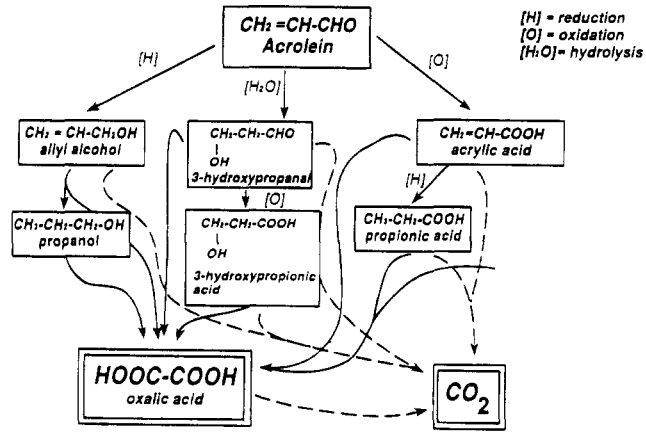

Figure 9. Production of oxalic acid and CO_2 for acrolein metabolism under anaerobic aquatic conditions comprised oxidative, reductive, and hydrolytic reactions. Under anaerobic or reducing conditions, the fate of the reductive product, allyl alcohol, was further elucidated by the formation of propanol.

Figure 9. The review of some aquatic toxicity literature and the degradation of acrolein in water suggest that toxicity concerns to aquatic organisms come from acrolein and not its metabolites.

2. *Distribution of Radioactivity.* After 30 days of anaerobic conditions, the ratio of radioactivity detected in the aqueous and sediment phases was comparable to the ratio observed in the 32-day aerobic study. By day 93 in the anaerobic systems, most of the remaining radioactivity, approximately 20% of the applied dose, was in the sediment phase. At study termination (day 178) 5% of the applied dose was in the aqueous phase and 11% was in the sediment phase. The overall decrease in total radioactivity observed in the aqueous phase was attributed both to sorption of acrolein degradation products to sediment solids and to their further mineralization to $^{14}CO_2$. Consequently, $^{14}CO_2$ was the major volatile product found in the volatile traps. Bound residues in the sediment phase were approximately 15% of applied radioactive dose at the termination of the 178-day anaerobic study. One-third of extractable sediment radioactivity (i.e., 4% of the applied dose) was determined to be bicarbonate anion, which indicated complete mineralization of acrolein degradates in the sediment phase as well as the aqueous phase of the test system.

ACKNOWLEDGMENT

We thank Jeffrey Bob and James E. Boliver of Springborn Laboratories, Inc., for generating the aerobic and anaerobic aquatic metabolism samples. We also thank Dr. Paul H. Fackler of Springborn Laboratories, Inc., for helpful advice.

LITERATURE CITED

Beauchamp, R. O.; Andjelkovich; Kiligerman, A. D.; Morgan, K. T.; Heck, H. A Critical review of the literature on acrolein toxicity. *CRC Crit. Rev. Toxicol.* **1985**, *14* (4), 309–380.

Boor, P. J. Allylamine cardiovascular toxicity: V. Tissue distribution and toxicokinetics after oral administration. *Toxicology* **1985**, *35*, 167–177.

Boor, P. J.; Sanduja, R.; Nelson, T. J.; Ansari, G. A. S. *In vivo* metabolism of the cardiovascular toxin, allylamine. *Biochem. Pharmacol.* **1987**, *36*, 4347–4353.

Bowmer, K.; Higgins, M. L. Some aspects of the persistence and fate of acrolein herbicide in water. *Arch. Environ. Contam. Toxicol.* **1976**, *5*, 87–96.

Brink, R. H., Jr. Studies with chlorophenols, acrolein, dithiocarbamates and dibromonitrilopropionamide in bench-scale biodegradation units, Betz Environmental Engingeering, Inc., Plymouth Meeting, PA. *Proceedings of the International Biodegradation Symposium 3rd Meeting Date*, **1976**; Sharpley, J. M., Kaplan A. M., Eds.; Applied Science: Barking, England, 1975; pp 785–791.

Brock, T. D. *Biology of Microorganisms*; Prentice-Hall: Englewood Cliffs, NJ, 1979; 410 pp.

Chou, W. L.; Speece, R. E.; Siddiqi, R. H. Acclimation and degradation of petrochemical wastewater components by methane fermentation. *Biotechnol. Bioeng. Symp.* **1978**, *8*, 391–414.

Draminski, W.; Eder, E.; Henschler, D. A new pathway of acrolein metabolism in rats. *Arch. Toxicol.* **1983**, *52*, 243–247.

Esterbauer, H.; Zollner, H.; Scholz, N. Reaction of glutathione with conjugated carbonyls. *Z. Naturforsch.* **1975**, *30*, 466–473.

Ghanayem, B. I.; Burka, L. T.; Matthews, H. B. Ethyl acrylate distribution, macromolecular binding, excretion, and metabolism in male fisher 344 rats. *Fundam. Appl. Toxicol.* **1987**, *9*, 389–397.

Hess, L. G.; Kurtz, A. N.; Stanton, D. B. Acrolein and its derivatives. *Kirk-Othmer, 3rd ed., Encyclopedia of Chemical Technology*; Mark, H. F., et al., Eds.; Wiley: New York, 1978; Vol. 1, p 277.

Izard, C.; Libermann, C. Acrolein. *Mutat. Res.* **1978**, *47*, 115–138.

Kaye, C. M. Biosynthesis of mercapturic acids from allyl alcohol, allyl esters and acrolein. *Biochem. J.* **1973**, *134*, 1093–1101.

Kincannon, D. F.; Stover, E. L.; Nichols, V.; Medley, D. Removal mechanisms for toxic priority pollutants. *J. Water Pollut. Control Fed.* **1983**, *55*, 157–163.

Mao, J.; Doane, R. A.; Kovacs, M. F. Separation of acrolein and its possible metabolites using different modes of high performance liquid chromatography. *J. Liq. Chromatogr.* **1994**, *17* (8), 1811–1819.

Patel, J. M.; Wood, J. C.; Leibman, K. C. The biotransformation of allyl alcohol and acrolein in rat liver and lung preparations. *Drug Metab. Dispos.* **1980**, *8*, 305–308.

Rikans, L. E. The oxidation of acrolein by rat liver aldehyde dehydrogenase. *Drug Metab. Dispos.* **1987**, *15*, 356–362.

Tabak, H. H.; Quave, S. A.; Mashni, C. I.; Barth, E. F. Biodegradability studies with organic priority pollutant compounds. *J. Water Pollut. Control Fed.* **1981**, *53*, 1503–1518.

Received for review December 23, 1994. Accepted June 14, 1995.[⊗] Funding of this study was provided by Baker Performance Chemicals, Inc.

JF9407275

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1995.