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# 2,3,6-Trichlorophenylacetic Acid (Fenac) Degradation in Aqueous and Soil Systems

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The herbicide fenac (2,3,6-trichlorophenylacetic acid) is an effective agent against broadleafs, grasses, and aquatic weeds (Gangstad 1972; Nat. Acad. Sci. 1968). The introduction of fenac into the aquatic environment for controlling indigenous plants is environmentally important because of its potential toxicity to aquatic fauna and flora and its possible adverse effects on man through soil and drinking water contamination. The herbicide and its degradation products may also accumulate in fish and pose additional health hazards. Furthermore, fenac may be converted to potentially more toxic and more persistent products than the parent chemical. Currently, little is known of the persistence and products of microbial metabolism of fenac in soil and aquatic environments.

The present study was thus undertaken to determine the environmental fate of this herbicide by studying its biodegradation and product formation in sewage, lake water, soil, and soil suspensions.

## MATERIALS AND METHODS

Uniformly ring-labeled $^{14}$C-fenac (2,3,6-trichlorophenylacetic acid) with a specific activity of 29 mCi/mmol was purchased from California Bionuclear Corp. (Sun Valley, CA). The radiochemical purity was >98% as determined by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC).

Technical grade fenac (70.9% of 2,3,6-trichlorophenylacetic acid) was provided by Amchem Products (Ambler, PA). This chemical is a mixture of trichlorophenylacetic acid isomers in which the 2,3,6-trichloro isomer constituted 71%. It was purified twice by recrystallization from hot benzene. The recrystallized material had a boiling point between 154-157°C and was 91% pure as determined by GLC.

All other chemicals used were reagent grade and purchased from Fisher Scientific Co. (Rochester, NY).

Gas chromatographic analysis was performed with a Hewlett-Packard Model 5840 GLC equipped with an electron-capture detector and a column containing 3% OV-1 on 100/120 mesh supelcoport (Supelco, Inc., Bellafonte, PA). The column was stainless steel and measured 1.83 m X 2 mm (i.d.). The column was operated isothermally at 165°C. The operating temperatures were 200°C for the injector and 325°C for the detector. The flow rate of the nitrogen carrier gas was 50 ml/min. The retention time of fenac was 3.0 min.

Analysis was also performed using a Waters Associate high-pressure liquid chromatograph (HPLC) Model 6000A equipped with Schoeffel SF 770 UV detector (at 225 nm) and a reverse phase column (μ-Bondapak C18). The column was eluted with a 60:40 (V/V) mixture of acetonitrile: 4% (V/V) acetic acid in water at 1.5 ml/min. Solvents were all spectgrograde and purchased from Baker Chemical Co. (Phillipsburg, NJ). The retention value was 8.5 ml.

Mass spectra were obtained at Cornell University (Ithaca, NY) with a Finnigan 3300 mass spectrometer, electron impact 70 eV coupled with a Finnigan 3300 gas chromatograph (GS/MS/DS) via a heated single-stage jet separator and using a glass column identical with the one described previously except that it was U-shaped and 1.53 m long.

All radioassays were performed using a Packard Tri-Carb Model 3255 liquid scintillation spectrometer equipped with automatic external standardization. All data were corrected for background interference, quenching, and counting efficiency. Aliquots of water and organic extracts were counted in Instagel (Packard Instrument Co., Inc.). $^{14}C$-fenac and metabolites eluting from the HPLC column were detected and quantified using a radioactivity flow detector (Radiometric Instrument and Chemical Co., Inc.) attached to the HPLC unit. Thin-layer chromatographs were scanned for radioactivity using a Nuclear Chicago Actigraph.

The biodegradation of fenac was determined using lake water and primary sewage effluent. Samples of freshwater were taken from Oneida Lake in Brewerton, NY. Fresh sewage was collected from a local domestic waste-water treatment plant and filtered to remove large particles. The chemical was added to 1 l of aqueous medium in sterile 2-l Erlenmeyer flasks to a final concentration of 2.0 mg/l. One set of flasks was amended with 500 mg of nutrient broth/l. The flasks were stoppered with foam plugs and incubated in the dark at 26 ± 1°C and shaking at 150 rpm. Sterilized water samples containing 2 mg fenac/l were used to assess non-biological degradation. Duplicate 5-ml aliquots were removed periodically and either mixed with 5 ml of acetonitrile, then filtered through 0.2-μm teflon filters (Milipore Corp., Bedford, MA) and analyzed for fenac and products by HPLC; or, acidified to pH 2, then extracted twice with diethyl ether, concentrated, and

derivatized with diazomethane (Woolson and Harris 1967) and analyzed by GLC or GLC/MS. The extraction efficiencies were 85-90%.

The mineralization of $^{14}$C-fenac was also determined in the aqueous media as well as in soil and soil suspensions. Samples (25 g) of locally-collected temperate soil (pH 6.9; organic matter 2.1%) were air-dried, passed through a 2-mm sieve and added to 250-ml biometer flasks (Bellco Glass, Vineland, NJ) containing 1.5 µg (1.1 µCi) $^{14}$C-fenac/g of soil. A portion (0.5 µg/g of soil) of unlabeled fenac was also added to give a final concentration of 2.0 µg/g of soil. The soil and chemical were mixed and distilled water added to bring the soil to 75% of field capacity. Soil suspensions were prepared from 100-g soil samples flooded with 250 ml of an inorganic salts solution as previously described (Rosenberg and Alexander 1980a). To 50-ml of either lake water, sewage, or soil suspension in biometer flasks was added 1.5 µg (1.1 µCi) of $^{14}$C-fenac and 0.5 µg of unlabeled fenac/ml. In an attempt to enhance mineralization of fenac, one set of flasks received 500 µg of nutrient broth either per g of soil or per ml of aqueous solution. The appropriate sterilized controls were used to assess non-biological degradation. All flasks were incubated in the dark at 26°C and 150 rpm. The sidearms contained 0.1 N KOH as the $^{14}CO_2$-trapping agent. The trapping agent was sampled (and replaced) periodically. The contents were verified as $^{14}CO_2$ by acidifying with 1.0 N HCl, and counted for radioactivity. Before the addition of 10 ml of fresh KOH, either 0.1 g of soil or 0.1 ml of liquid was removed, extracted as described, and 50-µl portions counted. At the termination of the experiment the contents in some of the biometer flasks were extracted and saved for GLC/MS.

## RESULTS AND DISCUSSIONS

The metabolism of fenac was first determined by using lake water and the primary effluent of municipal sewage. The degradation was followed by GLC and HPLC of extracted aliquots of the mixtures in the flasks. It is evident that the lake water and sewage differed in their activities (Fig. 1). In the nutrient-supplemented lake water, degradation was evident within one month and reached nearly 25% of the initial concentration of fenac (2 mg/l) after three months, while less than 7% of the initial fenac was degraded after 3 months in the unsupplemented water. In contrast, unsupplemented sewage had as much activity as supplemented lake water while supplemented sewage showed the loss of fenac within 1 week and after 3 months nearly 40% of the compound has been degraded. Less than 2% of the chemical was lost in sterile samples of lake water and sewage. At the end of the experiment the supplemented sewage was saved for GLC/MS analysis.

These findings are in contrast of those of others. Frank et al. (1963) studied the persistence of fenac in the water and hydrosoil

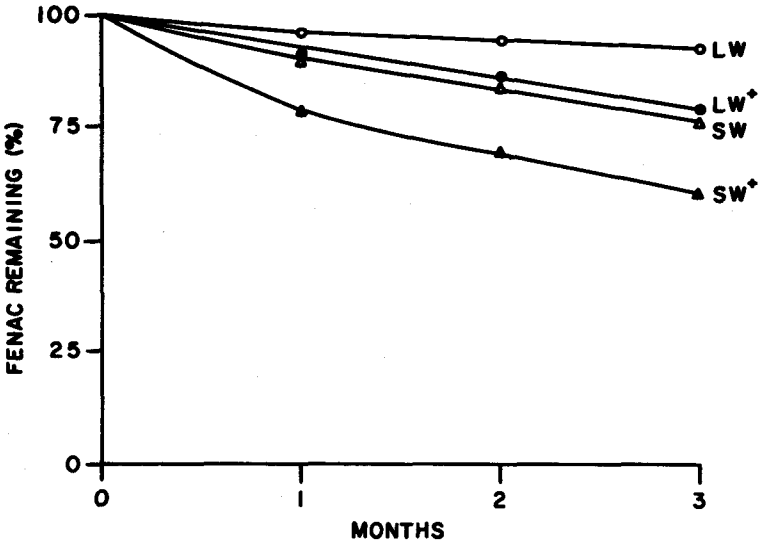


Fig. 1. Disappearance of fenac from lake water (LW), nutrient-supplemented water (LW+), sewage water (SW) and supplemented sewage water (SW+).

of two ponds treated with the herbicide at 1-1.56 parts per million (ppm). The herbicide residues could be detected in both the water (0.07-0.38 ppm) and sediment (0.08-0.26 ppm) 160 days after treatment. Similar negative results for lake water, sediment, and sewage were reported by Sikka et al. (1980). These disagreements with the present study may be attributed either to the absence of populations having the necessary catabolic enzymes, time of the year when the sample was taken, or nutrient depletion. The significantly enhanced activity in nutrient-supplemented lake water and sewage suggests that the fenac-degrading population proliferated and microbial activity increased at the expense of the readily available carbon and not at the expense of the herbicide. These results suggest that fenac may be degraded cometabolically with the cometabolizing species not replicating at the expense of the compound (Alexander 1979). Similar results for the herbicide 2,4,5-trichlorophenoxyacetic acid have been reported (Rosenberg and Alexander 1980a, 1980b).

The mineralization of uniformly ring-$^{14}$C-labeled fenac was also determined by measuring the evolution of $^{14}CO_2$ and the decrease of $^{14}$C-fenac in lake water, sewage, soil, and soil suspension (Fig. 2). Part A of Fig. 2 are data from the nutrient-supplemented flasks and part B are data from the unsupplemented flasks. $^{14}CO_2$ evolution and $^{14}$C-fenac disappearance are greater in

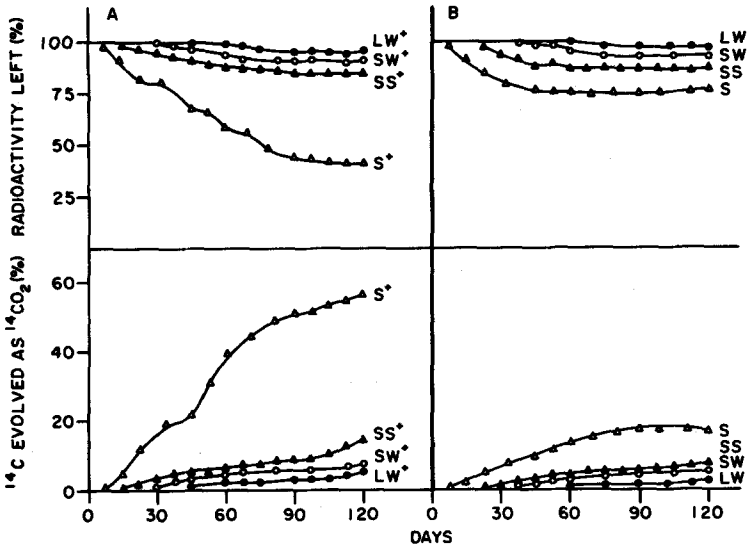


Fig. 2. Disappearance of fenac ($^{14}$C-ring-UL) (top) and evolution of $^{14}CO_2$ (bottom) from lake water (LW), sewage water (SW), soil suspension (SS) and soil (S) either nutrient supplemented (A) or not so amended (B).

nutrient-supplemented than in unsupplemented systems. The most dramatic effect was in supplemented soils where nearly 60% of the radioactivity was evolved as $^{14}CO_2$ and approximately 55% of the $^{14}$C-material had disappeared after 120 days. In contrast, in supplemented lake water nearly 60 days passed before $^{14}CO_2$ evolution was evident and only 5% was evolved after 4 months. Concomitantly, the loss of $^{14}$C-fenac was less than 5% after 4 months in the supplemented lake water. Thus, there are considerably different activities in the systems studied. These activity differences were not so evident in the unsupplemented systems, although the pattern of $^{14}CO_2$ evolution and $^{14}$C-fenac loss was similar to the supplemented systems. Because the sterilized systems showed less than 2% $^{14}CO_2$ release and chemical loss after 4 months and because the herbicide contained the label in the ring, the data confirm that the chemical underwent ring cleavage mediated by microbial activity only. At the end of the experiment the supplemented soil was extracted and saved for GLC/MS analysis.

Again, these data are in contrast to others who report the persistence of fenac in various soil types. Dowler et al. (1963) reported residues of fenac persisted for at least 2 years in coastal plain soils of North Carolina. Fenac was not degraded as measured either by the liberation of chloride ions after 3 to 4

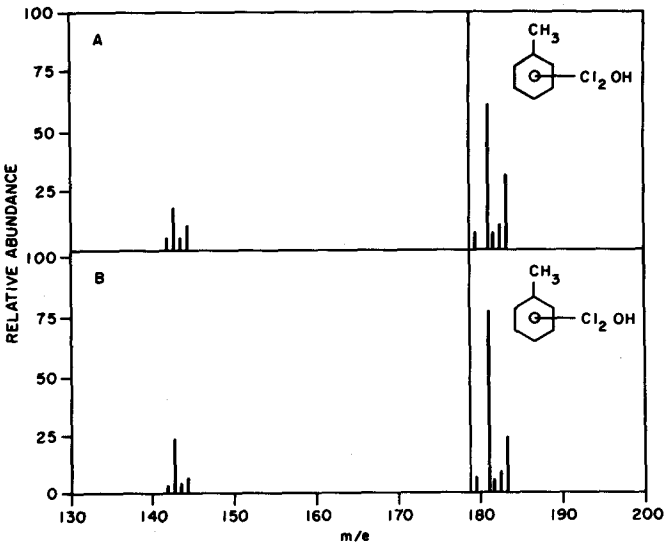


Fig. 3. Mass spectra of the methyl derivatives of a product from sewage water (A) and soil (B) incubated with fenac.

months in various soils (Bounds and Colmer 1965; Sheets et al. 1968) or by the evolution of $^{14}CO_2$ after 4 months (MacRae and Alexander 1965). However, failure to degrade a chemical does not preclude its biodegradability under more favorable conditions such as proper aeration, moisture, and nutrient content of the soils. Furthermore, adaptation by the degrading microbial population may take longer than the experimental time. If, however, the microorganisms cometabolize fenac then they will not proliferate at the expense of the chemical and the rate of degradation will remain low should the initial cell number be small. The addition of a readily degradable carbon source would allow replication of the cometabolizing population and enhance biodegradation of the herbicide as occurred in the supplemented soil and soil suspension.

The relatively low $^{14}CO_2$ evolution in lake water and sewage while exhibiting degradation of unlabeled fenac suggests a limited transformation to an aromatically-intact metabolite. A product was found in the extracts of the nutrient-supplemented sewage treated with unlabeled fenac (Fig. 3A) and supplemented soil treated with $^{14}C$-fenac (Fig. 3B). The derivatized extracts were analyzed by combined GLC/MS and produced identical mass spectra. The compounds had molecular ions with m/e of 177 and have been tentatively identified as a dehalogenated, hydroxylated toluene

derivative of fenac. The position of the hydroxyl group has not been determined, however; if it replaced the meta chloride group then the molecule may be more susceptible to ring cleavage. Such mechanisms for ring cleavage of both ortho and meta substituted metabolites have been shown for other herbicides and aromatic compounds (Rosenberg and Alexander 1980a; Horvath 1970; Gibson et al. 1968).

The data presented here indicate that fenac was degraded, albeit slowly in certain systems, and that this degradation is dependent on high cell densities and the ecosystem used. Herbicides with a chlorine in the meta position are generally resistant to microbial degradation (Burger et al. 1962) and apparently transformation of fenac to an aromatically-intact metabolite is easier than ring cleavage, at least in lake water and sewage. The identification of an aromatic metabolite with $^{14}CO_2$ evolution from soil suggest that this and/or other metabolites would not accumulate in soil because they would be mineralized by indigenous microorganisms.

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