

Microbial Degradation of Hydrochlorofluorocarbons (CHCl₂F and CHCl₂CF₃) in Soils and Sediments

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The ability of microorganisms to degrade trace levels of the hydrochlorofluorocarbons HCFC-21 and HCFC-123 was investigated. Methanotroph-linked oxidation of HCFC-21 was observed in aerobic soils, and anaerobic degradation of HCFC-21 occurred in freshwater and salt marsh sediments. Microbial degradation of HCFC-123 was observed in anoxic freshwater and salt marsh sediments, and the recovery of 1,1,1-trifluoro-2-chloroethane indicated the involvement of reductive dechlorination. No degradation of HCFC-123 was observed in aerobic soils. In some experiments, HCFCs were degraded at low (parts per billion) concentrations, raising the possibility that bacteria in nature remove HCFCs from the atmosphere.

Concerns about global warming and the integrity of stratospheric ozone have focused attention on eliminating the use of chlorofluorocarbons (CFCs) (18). A strategy has evolved of phasing in hydrochlorofluorocarbons (HCFCs) as temporary replacements while research on long-term replacements like hydrofluorocarbons is pursued (16). CFCs have long atmospheric residence times (60 to 100 years), whereas HCFCs and hydrofluorocarbons have residence times of ~15 years (23). The residence time of a halocarbon is one of several critical factors to be considered when assigning a value for its ozone depletion or global-warming potential (28). Residence times are calculated from the oxidation kinetics initiated by hydroxyl radicals and generally ignore the biosphere as an additional removal term (i.e., a global sink) for tropospheric halocarbons.

Lovley and Woodward (15) first demonstrated bacterial removal of near-atmospheric concentrations (i.e., mixing ratios) of CFC-11 (CCl₃F) and CFC-12 (CCl₂F₂) during incubation of anoxic soils, sediments, and bacterial cultures. Krone and Thauer (14) observed degradation of high concentrations of CFC-11 by *Methanosarcina barkeri*, and reductive dehalogenation was implicated. Removal of CFC-11 and CFC-113 (CH₃CF₃) in denitrifying groundwaters and dechlorination of CFC-11 in aquifer material undergoing sulfate reduction have been reported (25, 27). Vertical profiles of CFC-11 and CFC-12 in the anoxic waters of the Black Sea and Saanich Inlet suggest that biodegradation is occurring (3). Cumulatively, these reports make a case that anoxic ecosystems remove CFCs from the atmosphere. However, elsewhere, CFCs display conservative distribution patterns (4, 5, 8, 9, 12); hence, their biodegradation is confined to anoxic environments. Such restricted locales have a minor impact on the atmospheric residence times for CFCs (13).

In contrast, HCFCs are more reactive and should be more susceptible to bacterial attack. Metabolism of some HCFCs by methanotrophs has been reported (6, 11), and complete oxidation was implicated by the stoichiometric recovery of fluoride (11). However, no work has been done with soils or sediments to determine if the observations made with cultures can

be extrapolated to natural systems. In addition, the experiments with methanotroph cultures (6, 11) were conducted at very high concentrations (>1,000 ppm), whereas tropospheric mixing ratios of HCFC-21 are about 0.1 ppb (19, 24). It is not known whether soil microbes can degrade HCFCs present at low concentrations (e.g., parts-per-billion levels), let alone at tropospheric mixing ratios (e.g., sub-parts-per-billion levels). This study examined the ability of soils and sediments to metabolize HCFC-21 (CHCl₂F) and HCFC-123 (CHCl₂CF₃) and observed ongoing microbial degradation at concentrations as low as a few parts per billion.

Methanotrophic soils were taken from an agricultural field located in the Sacramento River delta, Calif. (20, 21). Soils (5 or 10 g, corresponding to volumes of ~5 to 10 ml) were dispensed into serum bottles (volume, 59 ml) and crimp-sealed with black butyl rubber stoppers under air. Methane and HCFC-21 (100 ppm) were added via a syringe. Controls consisted of heat-killed soils (autoclaved at 250 kPa and 121°C for 60 min), live soils incubated without O₂ (N₂ flushed for 10 min), and aerobic soils incubated without CH₄ additions. Soils were incubated statically at room temperature (~20°C). Additional CH₄ and/or O₂ was added during the incubations as required. Forest soil was recovered from the top 1.0 cm underlying the leaf litter layer in a deciduous forest near the U.S. Geological Survey headquarters in Reston, Va. Soil was dispensed (~95 g) into 180-ml serum bottles and sealed under air, and HCFC-21 (75 ppb) was injected. Anoxic sediments were taken from a salt marsh in southern San Francisco Bay (22) and a freshwater cattail marsh near Reston (15). Sediments (1 liter) were diluted with 250 ml of anoxic, sterile water and stirred thoroughly. The resulting dense slurry was passed through a funnel to remove rocks and sticks, and 95-ml volumes were dispensed into 180-ml serum bottles. All manipulations were performed in an anaerobic glove box. The serum bottles were capped, removed from the chamber, and flushed for 20 min with oxygen-free N₂-CO₂ (93:7). Heat-killed controls were autoclaved for 1 h on each of three consecutive days. Samples were given injections of HCFC-21 (70 to 75 ppb) or HCFC-123 (11.6 ppb), incubated statically in the dark at 20°C, and vigorously hand shaken for ~3 min prior to headspace samplings. An experiment with high levels of HCFC-123 (1.0 ppm) was run to detect dehalogenation products. All experiments were performed in triplicate, and the results are re-

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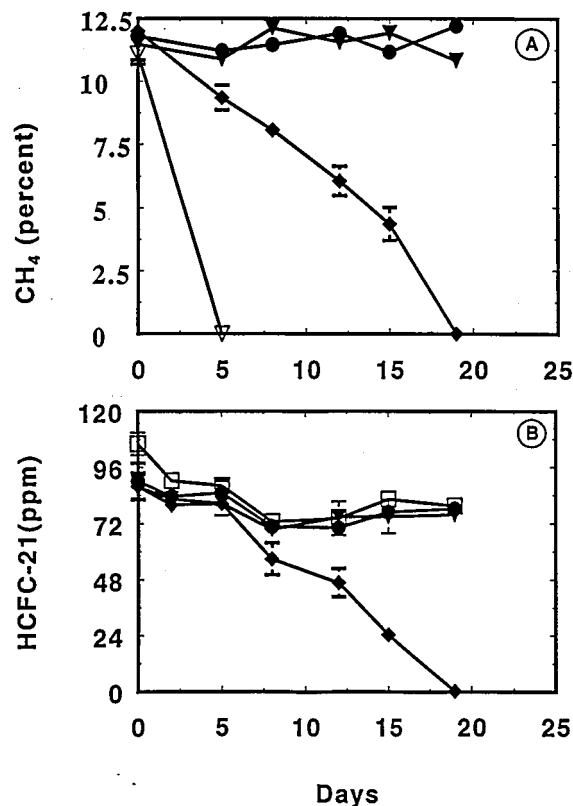


FIG. 1. Consumption of methane (A) and HCFC-21 (100 ppm) (B) by aerobic agricultural soils. Symbols: ▽, live without HCFC; ◆, live with HCFC; ●, live without O₂; ▼, killed; □, live without methane. Symbols represent the mean of three soil samples, and bars indicate ±1 standard deviation. The absence of bars indicates that the error was smaller than the symbol.

ported as the mean ± 1 standard deviation. The HCFCs were syringe sampled from the gas phases of samples and quantified by electron capture gas chromatography (15, 21). Detection limits and retention times were, respectively, 1.0 ppb and 5.7 min for HCFC-21, 0.05 ppb and 5.3 min for HCFC-123, and 1.0 ppb and 1.36 min for HCFC-133 (CF₃CH₂Cl). Methane was determined by flame ionization gas chromatography (15, 20, 21).

Aerobic agricultural soils oxidized both CH₄ and HCFC-21, whereas controls did not (Fig. 1). Oxidation of CH₄ by soils incubated without HCFC-21 was at least fivefold faster than oxidation of soils incubated with HCFC-21 (Fig. 1A), whereas soils without CH₄ did not degrade HCFC-21 (Fig. 1B). Therefore, methanotrophs were responsible for the HCFC-21 oxidation and HCFC-21 was obligately cooxidized with CH₄. The lower rate of CH₄ oxidation in the presence of HCFC-21 was due to the partial inhibition of methane oxidation by HCFC-21, as also occurs in *Methylococcus capsulatus* (17). Experiments with forest soils were performed with much greater quantities of soil than were the experiments with agricultural soils, so only about half of the HCFC-21 added was initially partitioned into the gas phase while the rest was presumably adsorbed onto the soil matrix (Fig. 2). Nonetheless, HCFC-21 was rapidly oxidized in live, aerobic samples whereas there was no loss in controls. This shows that bacterial oxidation of HCFC-21 in soils can occur rapidly and at concentrations which approach tropospheric mixing levels.

Anaerobic freshwater sediments rapidly degraded HCFC-21, whereas no significant loss occurred in the killed controls

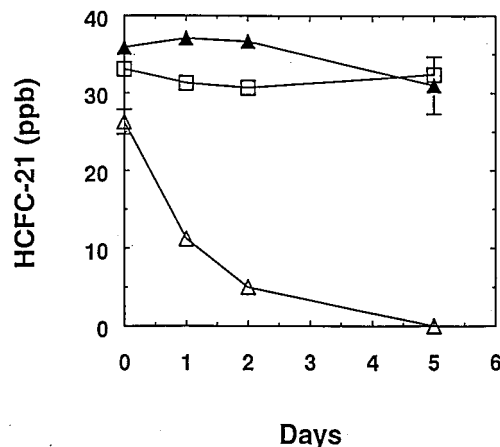


FIG. 2. Consumption of HCFC-21 (75 ppb) by aerobic forest soils. Symbols: △, live with O₂; ▲, killed; □, live without O₂. Symbols represent the mean of three soils, and bars indicate ±1 standard deviation. The absence of bars indicates that the error was smaller than the symbol.

(Fig. 3). Only a portion of the HCFC-21 (~21%) was initially partitioned into the vapor phase, because of large dissolved and adsorptive capacity of the dense slurry volume. Methane levels in the live samples increased from 8.7% ± 2.1% to 24.4% ± 3.8% after 27 days of incubation, whereas CH₄ was not detected in the killed controls (data not shown). About 1.0 ± 0.6 ppb of HCFC-21 persisted in the headspace of the samples, which would be equivalent to a total of about 5 ppb residual including the slurry phase. This indicates that 93% of the initially enclosed HCFC-21 was ultimately degraded. Results with salt marsh sediments incubated with 75 ppb of HCFC-21 differed from those with the freshwater sediments (results not shown). Initial levels of the HCFC-21 partitioned into the gas phases of live and killed controls were 24.7 ± 2.2 and 23.5 ± 3.6 ppb, respectively. After 8 days of incubation, these values did not change appreciably, and only after 43 days was there a significant difference between live materials (12.1 ± 1.7 ppb) and killed controls (17.4 ± 1.4 ppb). Hence, while live materials ultimately degraded ~50% of the HCFC-21, significant chemical degradation (~26%) also occurred in

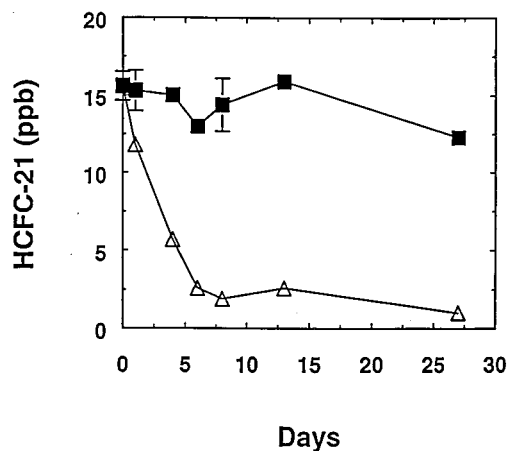


FIG. 3. Consumption of HCFC-21 (70 ppb) in anoxic freshwater sediments. Symbols: △, live; ■, killed. Symbols represent the mean of three soils, and bars indicate ±1 standard deviation. The absence of bars indicates that the error was smaller than the symbol.

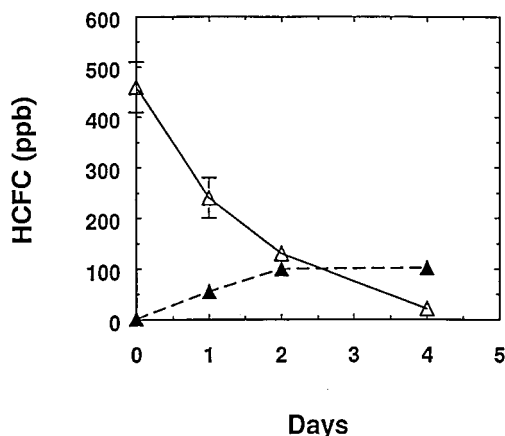


FIG. 4. Consumption of HCFC-123 (1 ppm) and production of 1,1,1-trifluoro-2-chloroethane by anoxic freshwater sediments. Symbols: Δ , HCFC-123; \blacktriangle , 1,1,1-trifluoro-2-chloroethane. Symbols represent the mean of three sediment slurries, and bars indicate ± 1 standard deviation. The absence of bars indicates that the error was smaller than the symbol.

the killed controls. Similarly, chemical degradation of CFC-11 has been observed (15).

We did not detect any loss of HCFC-123 (11.6 ppb) during prolonged incubation (43 days) of live or killed forest soils held under aerobic or anaerobic conditions (data not shown). However, anaerobic biodegradation of HCFC-123 occurred rapidly in both freshwater and salt marsh sediments (data not shown). About 61% of the HCFC-123 initially partitioned into the vapor phase. After 15 days of incubation of salt marsh sediments, live samples removed $\sim 82\% \pm 1\%$ of the HCFC-123 while killed controls removed $\sim 36\% \pm 1\%$. After 38 days, no HCFC-123 was detectable in live slurries whereas controls demonstrated no further loss. Biodegradation of HCFC-123 proceeded more rapidly in freshwater sediments, with live samples removing $96\% \pm 1\%$ after 3 days of incubation while killed controls removed only $38\% \pm 6\%$. No further loss of HCFC-123 occurred in controls even after 14 days of incubation. In addition, no HCFC-123 removal occurred in controls which contained only distilled water in lieu of either live or killed sediment slurries. Thus, a modest amount of chemical catalysis of HCFC-123 occurred in both freshwater and salt marsh sediments; however, biodegradation was clearly the predominant process. Although autoclaving may have decreased the chemical reactivity of the sediment matrix, the lack of any observable activity in the forest soils reasons against this argument. Biodegradation in freshwater sediments appears to have proceeded via reductive dechlorination, because we observed the accumulation of 1,1,1-trifluoro-2-chloroethane (Fig. 4). We did not conduct any further experiments to determine the fate of this compound.

The results of these studies show that trace levels (i.e., low parts per billion) of two HCFCs can be degraded quickly by the natural bacterial flora of certain soils and sediments. By comparison, oxidation of atmospheric methane (ambient mixing ratio, ~ 1.75 ppm) by soil bacteria is a well-characterized phenomenon (1) and is a significant global sink (2, 7, 10). This study has demonstrated that biological consumption of HCFC-21 will proceed at mixing ratios which are as much as 3 orders of magnitude below that of atmospheric methane (Fig. 2 and 3) and about 5 orders of magnitude below the HCFC levels used with methanotroph cultures (6, 11). DeFlaun et al. (11) reported oxidation of HCFC-21 but not HCFC-123 by *Methylosinus trichosporium*, and the results presented here agree with

their report. However, Chang and Criddle (6) demonstrated uptake of HCFC-123 by methanotrophic enrichments. Aerobic oxidation of HCFC-123 in soils remains to be demonstrated.

Even though the concentrations in the experiments described here were still higher than ambient mixing levels of HCFCs, they give credibility to the hypothesis that bacterial consumption will occur at the ≤ 0.1 -ppb level. Soils consume atmospheric methyl bromide, a halocarbon with an ambient mixing ratio (0.01 ppb) lower than that of most HCFCs (23, 26). A terrestrial sink equivalent to $\sim 20\%$ of the total atmospheric burden for methyl bromide was attributed to this bacterial activity, and the involvement of methanotrophs was not implicated (26). Although methanotrophs were clearly responsible for the aerobic incubations conducted with 100 ppm of HCFC-21 (Fig. 1), it is not certain if they caused the oxidation observed with 75 ppb of HCFC-21 (Fig. 2).

In summary, this study demonstrated that trace levels of HCFC-21 can be oxidized by bacteria in aerobic soils and that methanotrophs present a reasonable model for this activity. Anaerobic biodegradation of trace levels of both HCFC-21 and HCFC-123 was observed in anoxic sediments, and reductive dehalogenation is implicated. The slower degradation of HCFC-21 and HCFC-123 by anoxic salt marsh sediments contrasted with their rapid biodegradation in freshwater sediments, as well as with the biodegradation of HCFC-21 in the two aerobic soils. This suggests that considerable variability exists among the systems assayed with regard to their ability to metabolize HCFC-21 and HCFC-123. The fact that the activity observed generally occurred without lags suggests that natural populations of surficial soils and sediments may be adapted to the degradation of certain HCFCs. It also appears that HCFC-21 is more widely biodegradable by these bacteria than is HCFC-123. The ability of this microflora to consume HCFCs from the atmosphere and the global significance of this sink to all of the various types of HCFCs remain open to future experimentation.

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