Environmental Fate of the Next Generation Refrigerant 2,3,3,3-Tetrafluoropropene (HFO-1234yf)

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ABSTRACT: The hydrofluoroolefin 2,3,3,3-tetrafluoropropene (HFO-1234yf) has been introduced to replace 1,1,1,2-tetrafluoroethane (HFC-134a) as refrigerant in mobile, including vehicle, air conditioning systems because of its lower global warming potential. HFO-1234yf is volatile at ambient temperatures; however, high production volumes and widespread handling are expected to release this fluorocarbon into terrestrial and aquatic environments, including groundwater. Laboratory experiments explored HFO-1234yf degradation by (i) microbial processes under oxic and anoxic conditions, (ii) abiotic processes mediated by reactive mineral phases and zerovalent iron (Fe⁰, ZVI), and (iii) cobalamin-catalyzed biomimetic transformation. These investigations demonstrated that HFO-1234yf was recalcitrant to microbial (co)metabolism and no transformation was observed in incubations with ZVI, makinawite (FeS), sulfate green rust (GRSO₄), magnetite (Fe₃O₄), and manganese oxide (MnO₂). Sequential reductive defluorination of HFO-1234yf to 3,3,3-trifluoropropene and 3,3-dichloropropene with concomitant stoichiometric release of fluoride occurred in incubations with reduced cobalamins (e.g., vitamin B₁₂) indicating that biomolecules can transform HFO-1234yf at circumneutral pH and at ambient temperature. Taken together, these findings suggest that HFO-1234yf recalcitrance in aquifers should be expected; however, HFO-1234yf is not inert and a biomolecule may mediate reductive transformation in low redox environments, albeit at low rates.

INTRODUCTION

Chlorofluorocarbons, also known under the brand name Freon, were the most commonly used refrigerants until the 1980s, when their use was banned globally under the Montreal Protocol due to their ozone depletion effect. Hydrofluorocarbons (HFCs) were introduced as ozone-friendly alternatives, but came under scrutiny as potent greenhouse gases. HFC-134a was selected as the next generation refrigerant for MACs. The European Union’s top technical body, the Joint Research Centre, has recently concluded that HFC-1234yf is safe for use in automobiles, and the U.S. Environmental Protection Agency also encourages a rapid worldwide market transition from HFC-134a to HFC-1234yf. Several car manufacturers have since started to replace HFC-134a with HFO-1234yf. As a consequence of high-capacity use, future emissions of HFO-1234yf from MACs in Europe were estimated to range between 11.0 and 19.2 Gg yr⁻¹ after a complete conversion of the European fleet.

A few toxicological studies have been performed and HFO-1234yf demonstrated low potential for toxicity in rodents with no lethality observed at exposure levels of 2500 to 4000 ppm, but increased subacute inflammatory heart lesions were reported. In mammalian species, HFO-1234yf is subject to the typical fluoroolesin biotransformation pathway catalyzed by cytochrome P450 forming 2,3,3,3-tetrafluoroepoxypropene fol...
lowered by glutathione conjugation. Slightly different patterns in urinary metabolites have been reported due to the formation of species-specific glutathione S-conjugates.

Unlike saturated HFC refrigerants such as HFC-134a, HFO-1234yf is a fluoroolefin containing a more reactive C–C double bond, which may increase its susceptibility to (bio)chemical transformations. In the atmosphere, HFO-1234yf has an estimated lifespan of 10.5 days as it reacts with hydroxyl radicals (OH•) to form trifluoroacetyl fluoride, which hydrolyzes to trifluoroacetic acid (TFA). While the atmospheric fate of HFO-1234yf appears to be understood, its fate in terrestrial and aquatic (e.g., groundwater) ecosystems is unknown. At ambient temperatures, HFO-1234yf is volatile (boiling point −30 °C) and has an aqueous phase solubility of about 200 mg L−1 at 24 °C. While volatilization is a major pathway for HFO-1234yf entry into the environment, high production volumes and widespread handling are expected to introduce this hydrofluorocarbon into aquatic environments, including aquifers. The environmental fate of fluorinated hydrocarbons is far less understood than the degradation of chlorinated hydrocarbons, which has received ample attention. In general, carbon-halogen bonds can be broken by hydrolytic, oxygenolytic or reductive cleavage, and detailed knowledge about dechlorinating microorganisms and dechlorinating enzyme systems has been obtained. While many dechlorinases will also demobilize the fluorinated analogs are generally not attacked. This phenomenon has mainly been attributed to the strength of the C–F bond (CH3–F, 116 kcal mol−1; CH3–OH, 86 kcal mol−1; CH3–Cl, 81 kcal mol−1). Nevertheless, a number of bacteria use fluorinated hydrocarbons as growth substrates and possess enzyme systems that catalyze oxygenolytic, hydrolytic and reductive C–F bond cleavage. Cometabolic transformation and degradation of certain fluorooorganics also has been reported. For example, the degradation of hydrochlorofluorocarbons via methanotrophic cometabolism has been demonstrated in soils, mixed cultures, and pure cultures. While methanotrophic cometabolism is limited to aerobic environments, microbial cometabolic transformation can also occur under anaerobic conditions. For example, Neumann et al. and Schmitz et al. have previously shown that the PCE reductive dehalogenase (PceA) of Sulfovirgillum multivorans reductively dehalogenated halogenated propenes in vitro. Similarly, reduced transition metal cofactors, such as cob(1)-alams, can mediate reductive dehalogenation reactions.

Reactive mineral phases and zerovalent iron (ZVI) have also attracted attention for their roles in the degradation of halogenated pollutants in subsurface environments. A variety of Fe(II)-bearing mineral phases including mackinawite (FeS), green rust (GR), and magnetite (Fe3O4) as well as manganese oxide (MnO2) contribute to the degradation of different organic compounds, including organohalogens. ZVI walls and the injection of nano/micro-scale ZVI emulsions have been successfully implemented for in situ treatment of chlorinated solvent groundwater plumes.

To better understand and predict the fate of HFO-1234yf in aquatic and terrestrial environments, laboratory studies explored biotic and abiotic processes potentially contributing to HFO-1234yf transformation/degradation. Specifically, microbial degradation under aerobic and anaerobic conditions, abiotic degradation by reactive mineral phases and ZVI, and biomimetic reductive defluorination catalyzed by cob(1)alams were explored. These efforts suggested recalcitrance of HFO-1234yf in terrestrial and aquatic environments, although some reductive transformation to tri- and difluoropropenes may occur in low redox environments.

### Materials and Methods

**Chemicals.** HFO-1234yf (CAS#: 754–12–1) and 3,3,3-trifluoropropene (CAS#: 677–21–4) were purchased from Synquest Laboratories (>97%, Alachua, FL). Methane (CH4), tetrachloroethene (PCE) and trichloroethene (TCE) were purchased from Sigma-Aldrich (>99%, Saint Louis, MO), ACROS (>99%, Morris Plains, NJ), and Fisher Scientific (>99%, Pittsburgh, PA), respectively. ZVI was purchased from Alfa Aesar (>99%, Ward Hill, MA). Cyanocobalamin (vitamin B12, 98% purity) was purchased from Fisher Scientific (Fair Lawn, NJ), and hydroxocobalamin (95% purity), methyleneblamin (95% purity), adenosylcobalamin (98% purity), and titanium(III) chloride (15% solution in 10% HCl) were purchased from Sigma-Aldrich (Saint Louis, MO).

**Environmental Samples.** Sediment samples were collected from three geographically distinct locations (latitude, longitude), including the Third Creek in Knoxville, TN (35.949284, −83.939861), the Shady Valley in TN (36.519554, −81.933327) and the Axton Cross brown field site located alongside the Housatonic River in CT, USA (41.317139, −73.090358). The samples were aseptically transferred to sterile glass jars, which were completely filled, sealed and stored at 4 °C until use. The Third Creek and the Axton Cross sampling sites have a history of contamination with chlorinated solvents, whereas the Shady Valley location is considered as pristine water body.

**Aerobic Degradation and Methanotrophic Cometabolism.** Duplicate microcosms were established in 250 mL glass serum bottles closed with black butyl rubber stoppers (Geo-Microbial Technologies, Inc, Ochelata, OK). Each serum bottle received 5 g of sediment/soil (wet wt.) and mineral salts medium to achieve a total volume of 100 mL, and the headspace was filled with air as the source of oxygen. To evaluate if HFO-1234yf can be cometabolized by methanotrophs, duplicate serum bottles with and without CH4 were prepared. CH4 and HFO-1234yf were added aseptically using plastic syringes to achieve aqueous phase concentrations of 200 and 100 μM, respectively. Two additional bottles amended with CH4 and TCE served as positive controls for methanotrophic cometabolic activity. Neat TCE was added with a microliter glass syringe (Hamilton, Reno, NV) to achieve a final aqueous phase concentration of 10 μM. Autoclaved control microcosms with HFO-1234yf were established in duplicate for each set of live incubations. The serum bottles were incubated on a rotary shaker (120 rpm) at 20 °C, and CH4 and oxygen were monitored and replenished as necessary.

**Microbial Reductive Dehalogenation.** Duplicate microcosms were established in 160 mL glass serum bottles containing 100 mL reduced (Na2S·9H2O and i-cysteine, 0.2 mM each), bicarbonate-buffered (30 mM), defined mineral salt medium amended with 5 mM lactate and a N2–CO2 (80/20, v/v) headspace. Each bottle received 5 g of sediment material (wet wt.) and HFO-1234yf to achieve an aqueous phase concentration of 100 μM. Microcosms amended with 100 μM (aqueous phase concentration) PCE served as positive controls for reductive dehalogenation. HFO-1234yf was also tested for cometabolic reductive dehalogenation in microcosms that received PCE as electron acceptor together with HFO-1234yf.
Abiotic Degradation by Reactive Mineral Phases. To explore abiotic transformation, 10 g (dry wt.) of the reactive mineral phases and ZVI were added individually to duplicate 160 mL glass serum bottles containing 100 mL of deoxygenated 100 mM Tris buffer (pH 7.4). The bottles were sealed with butyl rubber stoppers. HFO-1234yf was added aseptically using a plastic syringe to achieve an aqueous phase concentration of 100 μM. Positive control incubations received neat TCE to achieve an initial aqueous phase concentration of 100 μM. MnO2 was demonstrated to react with bisphenol A (BPA), and MnO2-amended control bottles received 44 μM BPA. All bottles were incubated for 3 month horizontally on a rotary shaker (120 rpm) to prevent settling at 20 °C in the dark. The reactive minerals, except for ZVI, were synthesized in the laboratory following established procedures (see Supporting Information (SI) for details).

Abiotic Reductive Dechlorination by Cobalamins. Reductive dechlorination of HFO-1234yf and 3,3,3-trifluoropropene by titanium(III)-reduced cyanocobalamin was tested in triplicate 160 mL glass serum bottles with final volumes of 100 mL Tris buffer (100 mM, pH 7.4) and 60 mL N2 headspace. The bottles were wrapped with aluminum foil, and crimp sealed with black rubber stoppers. In addition to cyanocobalamin, cobalamins with different upper axial ligands, including hydroxocobalamin, methylcobalamin, and adenosylcobalamin were tested for reductive dechlorination of HFO-1234yf following the same procedure. Aqueous cobalamin stock solutions (1 mM) were prepared fresh, filter-sterilized and added to a final concentration of 10 μM. A 100 mM titanium(III) citrate stock solution was prepared as described and 5 mM were added to the vessels to initiate the reaction. All stock solutions were prepared using degassed and deionized water (Milli-Q Corporation, Bedford, MA), and the Hungate technique was followed to avoid oxygen contamination. Each vessel received 2.5 mL (ca. 100 μmol) of HFO-1234yf or 3,3,3-trifluoropropene (40 and 65 μM aqueous concentrations, respectively; see experimental determination of Henry’s constants below), and was incubated in 37 °C under static conditions in the dark. For quantification, triplicate standard curves were prepared by adding 10, 25, 50, and 100 μmol of HFO-1234yf and 1, 2.5, 5, and 10 μmol of 3,3,3-trifluoropropene to 160 mL serum bottles containing 100 mL of Tris buffer (100 mM, pH 7.4) (SI Figure S1). Triplicate control vessels lacked either cyanocobalamin and/or titanium(III) citrate and accompanied each experiment.

Analytical Procedures. CH4, TCE, PCE, HFO-1234yf, and 3,3,3-trifluoropropene were quantified using an Agilent 7890 gas chromatograph equipped with an Agilent model G1888A headspace autosampler, a flame ionization detector and a DB-624 capillary column (60 m × 0.32 mm × 1.8 μm). Aqueous phase (1 mL) and/or headspace (100 μL) samples were collected periodically for quantification of each compound. For all analytes, standard curves were prepared using known amounts of each compound in 160 mL bottles containing 100 mL of medium or Tris buffer (100 mM, pH 7.4). The formation of daughter products was confirmed using an Agilent 7890 gas chromatograph equipped with an Agilent 5975C mass selective detector and a DB-624 capillary column (60 m × 0.32 mm × 1.8 mm). Dimensionless Henry’s constants of 28.4, 35.0, 54.6, and 0.294 were used to calculate total amounts of CH4, PCE, and TCE, respectively, in the incubation vessels. The procedure described by Gossett was used to determine a Henry’s constants of 39.7 (±0.7, n = 3) and 22.8 (±0.4, n = 3) for HFO-1234yf and 3,3,3-trifluoropropene, respectively, at 20 °C. Values in the same range (i.e., 60 for HFO-1234yf and 23.9 for 3,3,3-trifluoropropene at 25 °C) were obtained using the U.S. Environmental Protection Agency’s Estimation Program Interface (EPI) Suite (http://www.epa.gov/optptintr/exposure/pubs/episuite.htm). Fluoride ions (F−) were monitored by ion chromatography using a Dionex ICS 2100 system equipped with a 4 mm × 250 mm IonPac AS18 hydroxide-selective anion-exchange column (Sunnyvale, CA). Oxygen was monitored using an Agilent 3000A Micro-GC with a Molecular Sieve 5A column and a thermal conductivity detector. X-ray diffraction (XRD) measurements were conducted using X'pert PRO (PANalytical Inc., Natick, MA) with Mo–Kα radiation at 60 kV/45 mA between 5° and 35° with a scan speed of 1.5° 2θ min−1. The specimen were mixed with acetone and stored inside an anoxic chamber until analysis. The slurry was smeared onto a silica zero background plate before measurement. Data were analyzed by profile fitting without any structural parameters using the JADE software package (Material Data Inc., Livermore, CA).

RESULTS

Aerobic Degradation. Underoxic conditions, HFO-1234yf degradation was not observed in any of the microcosms over a 3-month incubation period (data not shown). Inoxic microcosms amended with CH4, CH4 was consumed suggesting that methanotrophs were present and active, but the concentrations of HFO-1234yf did not decrease (Figure 1, SI Figure S2). In control vessels amended with TCE and CH4, TCE was degraded concomitantly with CH4 consumption in all the microcosms, presumably due to methanotrophic cometabolism. The CH4 oxidation rates diminished over time compared to the microcosms without TCE (Figure 1, SI Figure S2). No significant decreases in HFO-1234yf and CH4 concentrations were observed in the headspace of the negative...
control incubations suggesting that sorption to the sediment material and the rubber stoppers was negligible.

**Anaerobic Degradation.** In anoxic microcosms amended with 5 mM lactate as electron donor, reductive dechlorination or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control or degradation of HFO-1234yf were not observed during a 5-month incubation period (Figure 2, SI Figure S3). In control

![Figure 2. Concentration profiles of HFO-1234yf and PCE in microcosms incubated under anoxic conditions using sediment obtained from Third Creek. The results obtained from Shady Valley and Axton Cross are shown in the SI (Figure S2). Incubation conditions were HFO-1234yf only (○); PCE only (×); HFO-1234yf (▲) and PCE (△); HFO-1234yf (□); and PCE (■) with autoclaved sediment. TCE, cis-DCE, VC and ethene were observed as PCE dechlorination products (not shown). The error bars represent the range of duplicate microcosms. The notation aq. indicates aqueous concentration.](image)

microcosms amended with PCE, sequential reductive dechlorination to TCE, cis-1,2-dichloroethene (cis-DCE), vinyl chloride (VC) and ethene occurred, indicating organisms capable of reductive dechlorination were present and conditions conducive for this activity established in the microcosms. PCE reductive dechlorination was not affected by HFO-1234yf (Figure 2, SI Figure S3). Up to 45% of the initial amount of PCE disappeared in the negative (autoclaved) controls presumably due to sorption to sediment materials and the rubber stoppers, but no loss of HFO-1234yf occurred.

**Abiotic Degradation.** No HFO-1234yf transformation was observed in any of the incubations with reactive mineral phases or ZVI. In contrast, significant TCE degradation occurred in the positive control vessel over a 3-month incubation period (Table 1), indicating the iron-bearing minerals used in this study were reactive. XRD analysis confirmed that FeS and GRSO₄ were present in the expected phases (SI Figure S4). The TCE dechlorination rate constants and products with iron-bearing minerals and ZVI observed in this study and those reported in the literature are summarized in SI Table S1. Degradation of HFO-1234yf was also not observed in vessels amended with MnO₂, whereas positive control incubations were active and BPA was transformed (Table 1).

**In Vitro Reductive Defluorination by Vitamin B₁₂.** Reductive defluorination of HFO-1234yf with cyanocobalamin was observed in vessels amended with titanium(III) citrate, and a daughter product was formed. Based on GC–MS analysis and comparison with an authentic standard, the transformation product was identified as 3,3,3-trifluoropropene (Figure 3). No reduction of HFO-1234yf occurred in the absence of either cyanocobalamin or titanium(III) citrate suggesting that both cyanocobalamin and the reductant are required for reductive defluorination of HFO-1234yf. After 15 days of incubation, 10.7 ± 1.1 μmol of HFO-1234yf was reductively defluorinated to 10.9 ± 0.2 μmol of 3,3,3-trifluoropropene and 10.5 ± 0.4 μmol of fluoride was released (Figure 4). A pseudo-first-order

![Figure 3. Reduction of HFO-1234yf to 3,3,3-trifluoropropene (A) and 3,3,3-trifluoropropene to 3,3-difluoropropene (B) by vitamin B₁₂ (10 μM) with titanium(III) citrate (5 mM) as reductant. The error bars represent the standard deviation of triplicates. 3,3-Difluoropropene was not commercially available to prepare standard curves for quantification, and the peak area (pA*s) was used to demonstrate its formation from 3,3,3-trifluoropropene. TFP: 3,3,3-trifluoropropene, DFP: 3,3-difluoropropene.](image)

### Table 1. Reactivity of Mineral Phases and ZVI in Aqueous Batch Incubations (pH 7.4) a)

<table>
<thead>
<tr>
<th>reactive phase</th>
<th>reactant</th>
<th>degradation rate (L h⁻¹ g⁻¹)</th>
<th>products</th>
</tr>
</thead>
<tbody>
<tr>
<td>zero-valent iron (Fe⁰)</td>
<td>TCE</td>
<td>0.0066 (0.0016) b</td>
<td>ethane, butane</td>
</tr>
<tr>
<td>mackinawite (Fe₃O₄)</td>
<td>TCE</td>
<td>0.0053 (0.0018) b</td>
<td>acetylene</td>
</tr>
<tr>
<td>sulfate green rust (GRSO₄)</td>
<td>TCE</td>
<td>0.00049 (0.00036) b</td>
<td>acetylene</td>
</tr>
<tr>
<td>magnetite (Fe₃O₄)</td>
<td>TCE</td>
<td>0.00091 (0.00060) b *</td>
<td>—</td>
</tr>
<tr>
<td>manganese oxide (MnO₂)</td>
<td>BPA</td>
<td>37.7 (2.0) b *</td>
<td>HCA</td>
</tr>
</tbody>
</table>

* TCE and BPA served as positive controls for mineral phase and ZVI reactivity. The pseudo-first-order rate constants were normalized to the mass of the minerals. *Numbers in the parentheses represent the range of duplicate samples (i.e., max–min). *No detectable amount of products was observed. TCE: trichloroethene, BPA: bisphenol A, HCA: 4-hydroxycumyl alcohol.
rate constant of 0.00033 ± 0.00003 h⁻¹ \( (n = 3) \) was calculated for HFO-1234yf reductive defluorination. Under the same incubations conditions (10 μM cyanocobalamin and 5 mM titanium(III) citrate), PCE was reductively dechlorinated to TCE, cis-DCE and acetylene at a rate of 1.7 ± 0.5 h⁻¹ \( (n = 3) \). Hydroxocobalamin, methylcobalamin, and adenosylcobalamin also mediated HFO-1234yf reductive defluorination to 3,3,3-trifluoropropene at rates similar to those observed with cyanocobalamin (data not shown). Further defluorination of 3,3,3-trifluoropropene to 3,3-difluoropropene was not observed in the presence of HFO-1234yf; however, an independent experiment with 3,3,3-trifluoropropene (no HFO-1234yf present) demonstrated that reductive dechlorination to 3,3,3-trifluoropropene occurred in vessels amended with cyanocobalamin and titanium(III) citrate (Figure 3). 3,3-Difluoropropene was not commercially available but GC-MS analysis confirmed the formation of this compound (SI Figure S5). The removal of 11.6 ± 1.1 μmol 3,3,3-trifluoropropene coincided with the release of 10.4 ± 0.8 μmol of fluoride (Figure 3), indicating the stoichiometric reductive defluorination of 3,3,3-trifluoropropene to 3,3-difluoropropene, according to the pathway shown in Figure 4.

![Figure 4](image)

**Figure 4.** Sequential reduction defluorination of HFO-1234yf (2,3,3,3-tetrafluoropropene) to 3,3-difluoropropene by cyanocob(1)alamine.

#### DISCUSSION

The aerobic degradation of organic halogenated compounds is generally thermodynamically favorable \(^{48}\) and can potentially support microbial growth. The recalcitrance of some halogenated compounds has been attributed to the absence of suitable metabolic pathways and enzyme systems attacking the halogenated hydrocarbons. \(^{39}\) The potential for aerobic degradation generally decreases with the number of halogen substituitions. \(^{39}\) Consistent with this observation, mono- and dichlorohydrocarbons have been demonstrated to support microbial growth under oxic conditions. \(^{40,41}\) However, highly halogenated compounds often resist aerobic degradation.

Methane monooxygenase enzyme systems of methanotrophs share broad substrate specificities and are known to cometabolically oxidize over 300 different compounds, \(^{42,43}\) including several hydrofluorocarbons. \(^{14-16}\) The co-oxidation of TCE in the microcosms demonstrated methanotrophic activity but no HFO-1234yf loss occurred, suggesting that this hydrofluorocene is not susceptible to methanotrophic cometabolism. CH₄ oxidation rates were not affected by HFO-1234yf, whereas lower rates were measured in microcosms amended with TCE (Figure 1, SI Figure S2), consistent with TCE cometabolism. \(^{44}\)

Polyhalogenated hydrocarbons are generally more susceptible to reductive transformation under anoxic conditions, and thermodynamic calculations support that reductive defluorination reactions are associated with a substantial change in free energy. \(^{45,46}\) In none of the microcosms, reductive defluorination was observed, consistent with previous studies that failed to demonstrate reductive defluorination. \(^{14,47-49}\) The discovery of organochloride-respiring bacteria such as *Dehalococcoides mccartyi* has been challenging and it is possible that further efforts may discover specialized bacteria capable of reductive defluorination associated with energy conservation (i.e., organofluoride respiration).

The experiments with cob(I)alamins demonstrated that reductive defluorination of HFO-1234yf is feasible, and this process has also been demonstrated to work with perfluorooctanesulfonate (PFOS) branched isomers. \(^{51}\) The rate of HFO-1234yf reductive defluorination \((0.00033 ± 0.00003 \text{ h}^{-1})\) was 3 orders of magnitude slower than the rate of PCE reductive dechlorination under the same incubation conditions. Cob(I)alamins have been shown to reductively dehalogenate and transform carbon tetrachloride (CCL₄), dichlorodifluoromethane (CF₂Cl₂), and chlorotrifluoromethane (CF₃Cl) to carbon monoxide (CO). \(^{39}\) Cobalamins such as hydroxocobalamin, methylcobalamin, and adenosylcobalamin are biomolecules with a central cobalt atom that can be biologically reduced to the reactive Co²⁺ state. \(^{47,52}\) Therefore, no insurmountable barrier should exist that prevents microbes from catalyzing reductive defluorination reactions. A possible reason why organofluoride respiration has not been demonstrated may lie in the decade-long focus on chlorinated compound degradation and the relative scarcity of efforts to find deflorinators. The current study only tested sediment samples from three distinct locations. While the control experiments demonstrated the presence of methanotroph populations and organisms capable of reductive dechlorination, it is certainly possible that organofluoride-respiring bacteria are rare and not found in the sediments tested in this study. Therefore, the quest for microbes capable of reductive defluorination and organofluoride respiration should continue.

A relevant observation was the reductive defluorination of 3,3,3-trifluoropropene to 3,3-difluoropropene, suggesting that HFO-1234yf can be sequentially defluorinated. The trifluoromethyl group is considered recalcitrant to both chemical degradation and biological metabolism. \(^{53}\) Many aromatic fluorochemicals carry the trifluoromethyl group (e.g., the lampricide 3-trifluoromethyl-4-nitrophenol) and this group generally remains intact even after ring fission. \(^{54}\) Consistent with these observations, the trifluoromethyl group-containing compounds S-(3,3,3-trifluoro-2-hydroxypropyl)-mercaptacetic acid and N-acetyl-S-(3,3,3-trifluoro-2-hydroxypropyl)-l-cysteine were detected as predominant metabolites of HFO-1234yf biotransformation in urinary samples of mammalian species such as rats and rabbits. \(^{6-8}\) In vitro incubations of HFO-1234yf in liver microsomes from rats, rabbits and humans supported these observations. \(^{7}\) Further, photochemical reactions in the atmosphere transform HFO-1234yf to trifluoroacetate, a compound with a trifluoromethyl group, which returns to the surface during rain events. \(^{55,56}\) Our findings indicate that biomolecules (i.e., cob(I)alamins) can mediate C–F bond breakage suggesting that transformation of the stable trifluoromethyl group by biological systems may be feasible.

ZVI and metal-bearing minerals including FeS, GRSO₄, Fe₃O₄, and MnO₂ are known for their capacity to transform and degrade contaminants. \(^{22-31}\) A few studies have even demonstrated the abiogenic degradation of fluorinated organic compounds; \(^{30,36}\) however, we obtained no evidence for abiogenic degradation of HFO-1234yf by ZVI and metal-bearing minerals. The control experiments with PCE (ZVI, Fe₃O₄, GR₃O₄, Fe₃O₄) and BPA (MnO₂) demonstrated that the ZVI and the metal-bearing mineral phases were reactive. The TCE degradation rate constants measured for ZVI and Fe₃O₄ were more than 10-fold higher compared to those observed with GR₃O₄ and Fe₃O₄ (Table 1), consistent with rates reported in
the literature (SI Table S1). The particle size of the mineral phase and ZVI affects reactivity and it is possible that nanoparticulate materials (i.e., smaller particles) are more reactive; however, our findings suggest that abiotic processes involving ZVI, iron mineral phases and MnO$_2$ are unlikely to substantially contribute to the natural attenuation of HFO-1234yf in environmental systems.

In summary, the results of this study indicate that HFO-1234yf recalcitrance in oxic and anoxic subsurface environments (i.e., aquifers) should be expected. No evidence for microbial (co)metabolism was obtained, at least on the time scale of the performed laboratory experiments. Also, the abiotic transformation by ZVI and reactive minerals could not be demonstrated. Sequential reductive defluorination of HFO-1234yf to 3,3,3-trifluoropropene, and 3,3,3-trifluoropropene to 3,3-diﬂuoropropene by biomimetic reduction with cob(1)-alamin was observed in in vitro incubations. These findings suggest that HFO-1234yf is not inert and a biomolecule may mediate reductive transformation in anoxic environments, albeit at low rates.

**ASSOCIATED CONTENT**

3 Supporting Information

The Supporting Information details the synthesis of reactive mineral phases, compares reported TCE dechlorination rates with values obtained in the current study, presents standard curves for quantification of fluorinated propenes and mass spectra of 3,3-diﬁuoropropene, depicts microcosms results, and shows XRD patterns of mackinawite and green rust. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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