

Quantifying in-situ Transformation Rates of Chlorinated Ethenes by Combining Compound-Specific Stable Isotope Analysis, Groundwater Dating, and Carbon Isotope Mass Balances

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Abstract

We determined *in-situ* reductive transformation rates of tetrachloroethene (PCE) in a contaminated aquifer by combining compound-specific carbon stable isotope analysis (CSIA) of the contaminants with tracer-based (^3H - ^3He) groundwater dating. With increasing distance from the source, PCE was gradually transformed to trichloroethene (TCE), *cis*-dichloroethene (cDCE), and vinyl chloride (VC). Using an *in-situ* determined carbon isotopic enrichment factor of $-3.3 \pm 1.2\%$ allowed for quantification of the PCE-to-TCE transformation based on isotopic ($\delta^{13}\text{C}$) shifts. By combining these estimates of the extent of PCE transformation with measured groundwater residence times (between 16 and 36 years) we calculated half-lives of 2.6 ± 0.8 years ($k = 0.29 \pm 0.09 \text{ yr}^{-1}$) for the PCE-to-TCE transformation. Carbon isotope mass balances including the chloroethenes PCE, TCE, cDCE and VC ($\delta^{13}\text{C}_{\Sigma(\text{CEs})}$) enabled an assessment of complete PCE dechlorination to non-chlorinated products. Shifts of $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ at the fringe of the plume of more than 25‰ pointed to dechlorination beyond VC of up to $55 \pm 17\%$ of the chloroethene mass. Calculated rates for this multi-step dechlorination were highly variable throughout the aquifer ($k = 0.4 \pm 0.4 \text{ yr}^{-1}$), suggesting that PCE reduction to non-chlorinated products occurred only in locally restricted zones of the investigated site.

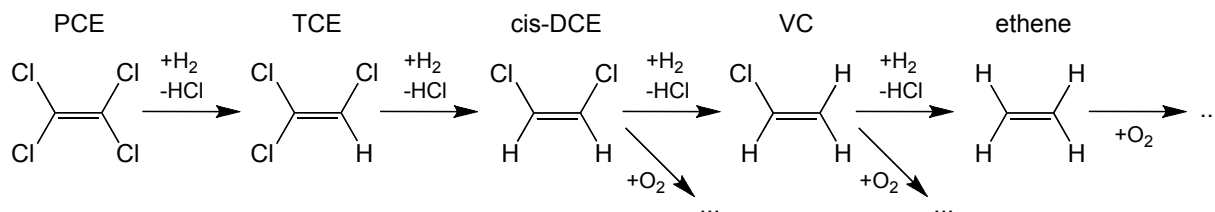
Introduction

Groundwater pollution caused by chlorinated ethenes (CEs) is a widespread problem in industrial areas (1). Because of their slow degradation in aquifers, tetrachloroethene (PCE) and trichloroethene (TCE) may persist for decades in the subsurface, in many cases as dense non-aqueous phase liquids (DNAPLs). Nevertheless, *in-situ* attenuation of CEs by naturally occurring (or stimulated) (bio)transformation is often the only remediation option owing to the lack of successful alternative strategies for *ex-situ* treatment (2). Therefore various conceptual approaches (and combinations thereof) are being developed to assess degradation processes in the subsurface reliably and quantitatively. While one of the most promising recent approaches to evaluate the *extent* of organic pollutant transformation is

compound-specific stable isotope analysis (CSIA) (3, 4), information on the *time scales* of such processes can, in principle, be obtained from transient tracer based groundwater dating (5).

Changes of the carbon isotope composition in CEs observed with increasing distance from a spill site can reveal both the occurrence as well as the pathway(s) of degradation (6-11). Under anoxic conditions, the enzyme-catalyzed sequential hydrogenolysis of PCE or TCE via *cis*-dichloroethene (cDCE) and vinylchloride (VC) to ethene is the only microbial pathway to yield transformation products of no (eco-)toxicological concern (12). In the presence of oxygen, in contrast, cDCE and VC can also be mineralized microbially via oxidative pathways (Scheme 1) (13).

Scheme 1



To assess the extent of transformation of CEs and to distinguish between competing reaction pathways of less chlorinated products, bulk carbon and chlorine isotope enrichment factors (ϵ) have been determined for the different dechlorination steps (see, e.g., (14, 15) and refs cited therein). These ϵ -values are considered indicative for a transformation process because they reflect the isotope fractionation caused by a bond-specific kinetic isotope effect (16).

However, the complex kinetics of chloroethene transport to the enzymatic site of reaction (e.g., due to (D)NAPL dissolution) and/or specific enzyme-substrate interaction in different dehalorespiring organisms of CEs can cause isotope enrichment factors to vary significantly despite identical reaction mechanisms ((15, 17), see Table S3 in the Supporting Information (SI) for a compilation of ϵ_C -values). Such variability in isotope enrichment behavior likely compromises the applicability of CSIA. As in the case for PCE- and TCE-contaminated sites, natural attenuation is only achieved after complete dechlorination, that is, if none of the toxic products such as VC accumulates. In fact, assessing the

extent of reaction within the entire PCE hydrogenolysis sequence to ethene with uncertain isotope enrichment factors has been recognized as a major challenge (see, e.g., ref (9)).

The general objective of this study was therefore to advance the applicability of stable isotope analysis as a tool to evaluate if and to what extent complete dechlorination of PCE occurs as well as to provide estimates for the rates of this multistep process. To this end, we carried out a field study, in which rates of *in-situ* hydrogenolysis of PCE to TCE and of the complete dechlorination were calculated from the combined use of compounds-specific carbon isotope analysis of CEs and groundwater dating with the ^3H - ^3He method (18). The latter is the method of choice for slow dechlorination processes of CEs in subsurface environments which exhibit half-lives of years to decades. In addition to a quantitative evaluation of single reductive dechlorination steps from the carbon isotope enrichment of individual chloroethenes, we studied the changes of the concentration-weighted average of PCE, TCE, cDCE, and VC isotope signatures, $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ (isotope mass balance), for quantification of the sequential hydrogenolysis of PCE to ethene. This mass balance procedure was previously applied at a chlorobenzene-contaminated field site, albeit data interpretation was only carried out qualitatively (19). As will be outlined below for the natural attenuation of PCE in the field, this approach is nevertheless sensitive to the variability and thus to the choice of most appropriate ε -values for the individual reductive dechlorination steps. From a practical perspective (and for equal transport behavior of all CEs), however, a constant $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ points towards partial CE reduction to chlorinated products only, whereas deviations of $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ from the $\delta^{13}\text{C}$ value of the CE contamination source are a clear indication of successful microbial dechlorinating activity beyond the accumulation of toxic products.

Material and Methods

Description of Field Site. Figure 1A depicts the field site where a dry-cleaning facility operated from the mid-1940ies to 1987. An average monthly PCE consumption of 1000–2000 L is reported. A major leakage of PCE to the subsurface happened in 1969. Following the removal of the facility, new buildings

were constructed without excavation of the contaminated soil. The geology is characterized by two vertically stacked aquifers, separated by a 5–20 m thick clay aquitard (Figure 1B). The upper, contaminated aquifer is formed by 8–12 m of clean silty sand and gravel of glaciofluvial deposition, and is covered by a confining layer of silty clay with a thickness of 2–5 meters. This aquifer was equipped with 36 monitoring wells, six of them tapping the aquifer at two different depths. Low hydraulic conductivities of the aquifer ($1\text{--}8 \times 10^{-5}$ m/s; determined by flow-meter tests) and an average hydraulic gradient of 0.3% lead to Darcy groundwater velocities of only 1–8 m/yr.

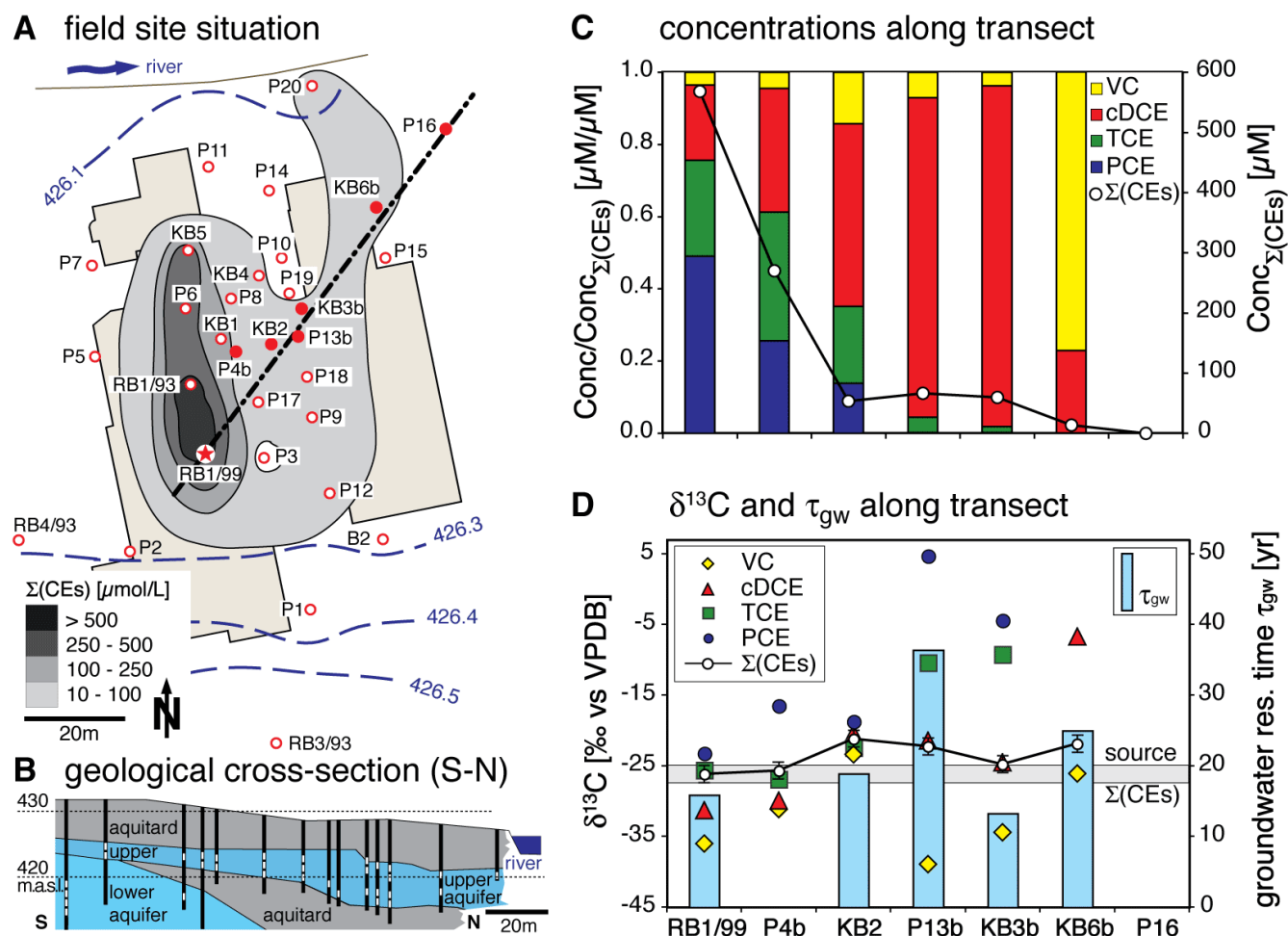


Figure 1. **A)** Map of the field site indicating the 37 monitoring wells (circles), present buildings (brown shapes), and groundwater heads (dashed lines; in m asl). Full circles are monitoring wells along the intensively investigated transect (dashed-dotted line). The gray shaded areas depict the plume of total CE groundwater concentrations (sum of PCE, TCE, cDCE and VC). **B)** Geological S-N cross-section of field site, including the position and screens of the boreholes. The investigated upper aquifer, the lower aquifer, and the confining layers (aquitard) are shown. **C)** Total CE concentrations (circles, right y-axis)

and single CE concentrations normalized by the total CE concentration (bars, left y-axis) along the transect. **D)** Carbon isotopic signatures of CEs (symbols, left y-axis) and measured groundwater residence times τ_{gw} (columns, right y-axis). The solid line represents the isotopic mass balance $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ considering all CEs. The horizontal bar indicates $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ in the source well ($-26.1 \pm 1.2\%$). Concentration and isotopic signatures were below detection limit ($<0.05 \mu\text{mol L}^{-1}$) in the last well of the transect (P16).

Groundwater Sampling. Groundwater wells were purged with submersible pumps (MP1, or Gigant, Grundfos, Bjerringbro, Denmark) at least three times the well volumes and until electric conductivity, dissolved oxygen, pH and redox potential reached stable readings. Samples were collected with a peristaltic pump (Type P2.52, Eijkelkamp, Giesbeek, Netherlands) through PTFE hoses. To avoid cross-contamination between sampling points, the short silicon tube in the peristaltic pump was replaced for each sampling point. Water samples for CE concentrations and isotopic analyses were stored in 120 mL glass bottles which were slowly filled, sealed without headspace using PTFE-lined screw caps and stored in the dark at 4 °C until analysis, performed within four weeks after sampling. Samples for groundwater dating were collected and sealed in copper tubes as described elsewhere (20). For analysis of major anions, samples were stored in polyethylene bottles. Aliquots for analysis of dissolved Fe and Mn were filtered on-site ($0.45 \mu\text{m}$) and acidified ($\text{pH} < 2$ using HNO_3).

Analytical Methods. CE concentrations were quantified using direct aqueous injection-GC/MS (21) with typical accuracy of $\pm 10\%$. Concentrations of major anions (Cl^- , NO_3^- , SO_4^{2-}) were analyzed by ion chromatography; ammonium by photometry; Fe and Mn by ICP-OES. Dissolved hydrogen sulfide was quantified on-site using a colorimetric test kit (Aquaquant, Merck). Groundwater residence times (uncertainty ± 1 yr) were determined with the ^3H - ^3He method that is based on quantification of ^3He originating from precipitation-derived radioactive tritium (18, 20).

Compound-specific stable carbon isotopic signatures of CEs were measured using a GC (Trace GC 2000; Thermo Scientific, Bremen, Germany) equipped with a 60 m long Rtx-VMS column (0.32 mm I.D., 1.8 μm film, Restek, Bellefonte, PA, USA) coupled to an isotope ratio mass spectrometer (IRMS, Delta V, Thermo Scientific) via a combustion interface (GC Combustion III, Thermo Scientific) maintained at 940 °C. A slight modification of the P&T-GC-C-IRMS method described in (22) was

applied (details in SI). After a set of ten measurements, the system stability was verified by analyzing standard solutions containing the four CEs of known isotopic composition and at concentration ranges matching the samples. The deviation in $\delta^{13}\text{C}$ -values for duplicate measurements was always $<1\text{‰}$. With this procedure, the uncertainty of CSIA-analysis can be estimated as $\pm 0.5\text{‰}$ (23). All isotopic ratios R ($^{13}\text{C}/^{12}\text{C}$) are reported relative to the international standard Vienna PeeDee Belemnite (VPDB) and in the delta notation, given by:

$$\delta^{13}\text{C}_{PCE} = (R_{\text{sample}} / R_{\text{VPDB}} - 1) \cdot 1000\text{‰} \quad (1)$$

Data Analysis. A Rayleigh approach (eq 2) was used to quantify PCE biodegradation in the aquifer:

$$1 - B_{PCE} = \left(\frac{\delta^{13}\text{C}_{PCE} + 1000\text{‰}}{\delta^{13}\text{C}_{PCE}^{\text{source}} + 1000\text{‰}} \right)^{1000\text{‰}/\epsilon_{PCE}} \quad (2)$$

where $\delta^{13}\text{C}_{PCE}$ and $\delta^{13}\text{C}_{PCE}^{\text{source}}$ are the isotopic signatures of PCE in the contamination plume and source, respectively. The term $(1 - B_{PCE})$ represents the fraction of PCE that has not been subject to biodegradation (24), and $(1 - B_{PCE})$ is usually determined from the relative aqueous concentrations of PCE in the plume vs. its source concentration. In the environment, however, changes in aqueous PCE concentration are not only affected by biodegradation but also by non-degradative physical processes such as sorption and dilution. Since the investigated glaciofluvial sandy aquifer typical has a low fraction of organic carbon (f_{OC}) of 0.1-1‰ (25), sorption is likely unimportant, and retardation factors of PCE and TCE between 1.0 and 1.8 have been calculated for a nearby aquifer of the same geological formation (26). To account for dilution, estimates of the fraction of nondegraded PCE, that is $(1 - B_{PCE})$, were obtained using eq 3:

$$1 - B_{PCE} = \frac{x_{PCE}}{x_{PCE}^{\text{source}}} \quad (3)$$

where x_{PCE} is the mass fraction of PCE relative to the total mass of PCE and its degradation products. For samples with dechlorination occurring not further than VC, x_{PCE} can be expressed by the aqueous CE concentrations (eq 4):

$$x_{PCE} = \frac{[PCE]^{aq}}{[\Sigma(CEs)]^{aq}} \quad (4)$$

where $[PCE]^{aq}$ and $[\Sigma(CEs)]^{aq}$ are the aqueous PCE and total CE concentration, at a certain well, respectively. Eqs 3-4 were inserted in the Rayleigh equation (eq 2) in order to determine *in-situ* ε_{PCE} -value (see Results and Discussion).

As a consequence of the sequential reductive dechlorination of CEs, the weighted average of the CEs isotope signature, $\delta^{13}C_{\Sigma(CEs)}$ (eq 5), remains constant if VC is not further transformed. On the other hand, $\delta^{13}C_{\Sigma(CEs)}$ increases beyond the source $\delta^{13}C$ -value of PCE in the case of further dechlorination of VC to ethene and other non-chlorinated products.

$$\delta^{13}C_{\Sigma(CEs)} = \delta^{13}C_{PCE} \cdot x_{PCE} + \delta^{13}C_{TCE} \cdot x_{TCE} + \delta^{13}C_{cDCE} \cdot x_{cDCE} + \delta^{13}C_{VC} \cdot x_{VC} \quad (5)$$

See Section S4 in SI for derivation of eq 5. The terms x_{TCE} , x_{cDCE} and x_{VC} were defined in analogy to eq 3. Note that for the case of significant sorption of the CEs to the aquifer sediments, differences in advective flow of individual CEs can also cause $\delta^{13}C_{\Sigma(CEs)}$ to vary (27). However, this is a local phenomenon at the front of the plume due to distinct retardation factors for each CE, which is not conceivable any more in a steady state situation. Therefore, deviations of $\delta^{13}C_{\Sigma(CEs)}$ are generally indicative for the potential of natural attenuation processes, i.e. for complete dechlorination of PCE.

In analogy to eq 2, the degree of total CEs transformation to non-chlorinated compounds is:

$$B_{\Sigma(CEs)} = 1 - \left(\frac{\delta^{13}C_{\Sigma(CEs)} + 1000}{\delta^{13}C_{\Sigma(CEs)}^{src} + 1000} \right)^{1000/\varepsilon_{\Sigma(CEs)}} \quad (6)$$

where $\varepsilon_{\Sigma(CEs)}$ is the isotopic enrichment factor associated with the complete PCE dechlorination. Due to masking of single-step isotopic fractionation within a multi-step reaction, $\varepsilon_{\Sigma(CEs)}$ -values are influenced by the kinetics of the individual reaction steps (see, e.g., ref. (28)). As derived in the SI Section S4, the minimum and maximum limits of $\varepsilon_{\Sigma(CEs)}$ -values can still be estimated from enrichment factors of individual dechlorination steps:

$$\varepsilon_{\Sigma(CEs)}^{max} \approx \varepsilon_{PCE} + \varepsilon_{TCE} + \varepsilon_{cDCE} + \varepsilon_{VC} \quad (7)$$

$$\varepsilon_{\Sigma(CEs)}^{min} \approx \varepsilon_{VC} \quad (8)$$

Eq 7 represents the limit of instantaneous transformation of PCE to VC whereas eq 8 is valid for the limit of a significantly slower transformation of VC compared to the preceding reaction steps. Based on data from this study and from literature, $\varepsilon_{\Sigma(CEs)}^{min}$ and $\varepsilon_{\Sigma(CEs)}^{max}$ values were -22‰ and -51‰, respectively (see Results and Discussion).

The time scale of CE biodegradation was assessed based on measured groundwater residence times, τ_{gw} . Because the aquifer is confined by aquitards at the top and the bottom, we inferred that all water packages downstream of the source passed along the source zone (PCE dissolution from DNAPL) where their groundwater residence time was τ_{gw}^{source} . In order to track the same water packages we collected groundwater from the lowest 1.5–2 m of the aquifer whenever possible. Therefore, pseudo-first-order degradation rates (k_{PCE}) were calculated for the one-step PCE dechlorination:

$$\ln(1 - B_{PCE}) = -k_{PCE} \times (\tau_{gw} - \tau_{gw}^{source}) \quad (9)$$

For the complete PCE dechlorination, an overall degradation rate constant ($k_{\Sigma(CEs)}$) was calculated in analogy to eq 9 as:

$$\ln(1 - B_{\Sigma(CEs)}) = -k_{\Sigma(CEs)} \times (\tau_{gw} - \tau_{gw}^{source}) \quad (10)$$

Results and Discussion

Qualitative Evidences of PCE Dechlorination. As is shown in Figure 1A, the groundwater exhibited high concentrations of PCE (280 $\mu\text{mol L}^{-1}$) and its less chlorinated metabolites TCE, cDCE and VC (20–150 $\mu\text{mol L}^{-1}$) at the former PCE tank location (well RB1/99), forming a contaminant plume towards the northeast. DCE isomers other than cDCE, that is *trans*-1,2- and 1,1-dichloroethene, accounted for <3% of the cDCE concentration and were thus not considered in the assessment. The presence of

hydrogenolysis products is a qualitative evidence for reductive PCE biodegradation, as PCE was the only solvent used on site. This is in agreement with the observed gradual dechlorination pattern along the transect depicted in Figure 1C, where cDCE and VC become abundant with increasing distance from the source. Moreover, cDCE and VC were distributed over larger spatial extensions than PCE (see Figure S1 in SI). Reductive transformation of PCE is favored by reducing groundwater conditions present in most of the wells (low O_2 , NO_3^- and SO_4^{2-} , and elevated Fe and Mn concentrations; see Table S2 in SI). Under such conditions, the oxidative transformation of cDCE and VC are likely hampered (13).

Extent and Rate of *In-Situ* PCE Dechlorination. To quantify the extent of PCE transformation, the $\delta^{13}C$ of the CEs were investigated. Together with a change in CE concentration patterns, increasing $\delta^{13}C$ values of PCE, TCE, cDCE, and VC were observed along the studied transect in a manner that is characteristic for a series of sequential reactions (Figure 1D). We applied a dilution-corrected Rayleigh-equation according to eqs 2-4 to calculate a bulk ^{13}C enrichment factor (ϵ_{PCE}) for PCE hydrogenolysis to TCE of $-3.3 \pm 1.2\%$ for the field conditions (Figure 2A). In this evaluation we included only the subset of 9 samples where degradation did not proceed beyond VC, i.e. that exhibited a constant isotopic mass balances over all CEs ($\delta^{13}C_{\Sigma(CEs)} = -26.1 \pm 1.2\%$, i.e. $B_{\Sigma(CEs)} < 5\%$, see Table 1 and S1 and discussion below for details), and corrected for dilution. The obtained ϵ_{PCE} value, which is in the range of published data for PCE dechlorination by mixed cultures from contaminated field sites under laboratory conditions (-2 to -7% , Table S3 in the SI), was then used to assess degradation of the comprehensive set of samples.

Using this site-specific ϵ_{PCE} -value, the extent of PCE transformation to TCE (B_{PCE}) was calculated according to eq 2 for all samples (Tables 1 and S1). The results show that PCE was transformed efficiently in most of the wells ($B_{PCE} > 60\%$ in 28 of 31 wells). PCE was even completely transformed ($B_{PCE}=100\%$) in 14 wells, and of the three wells with insignificant PCE transformation ($B_{PCE} < 20\%$), two are situated in the source zone (RB1/99 and RB1/93).

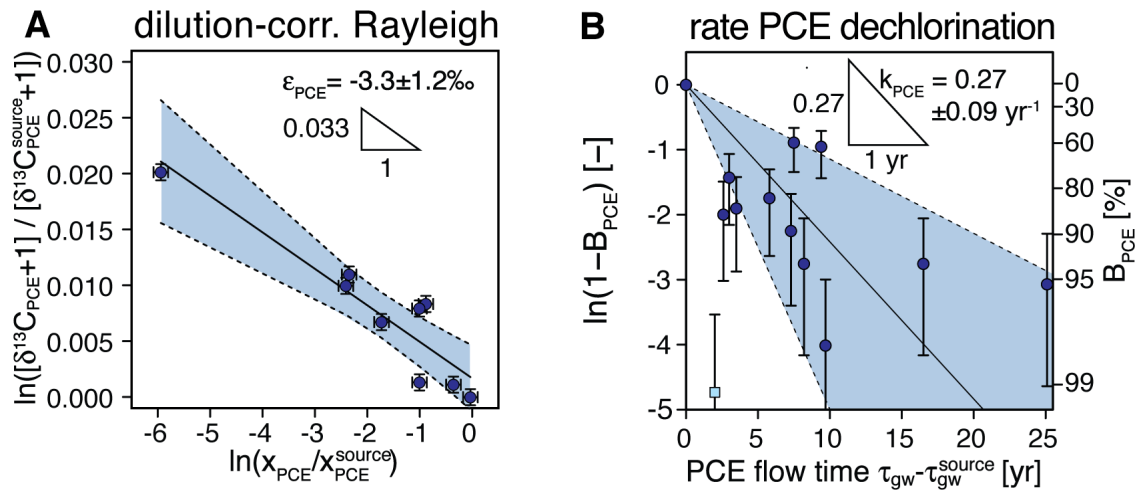


Figure 2. **A)** Evaluation of field site-specific ϵ_{PCE} -value for PCE-to-TCE transformation, based on PCE concentrations (normalized by the total CE concentration to correct for dilution) and $\delta^{13}\text{C}$ -values. A uniform isotopic enrichment with $\epsilon_{\text{PCE}} = -3.3 \pm 1.2\text{‰}$ resulted (95% confidence). **B)** Pseudo-first-order degradation rates derived from isotope-based degrees of degradation B_{PCE} and groundwater residence times τ_{gw} . The corresponding rate constants k_{PCE} is $0.27 \pm 0.09 \text{ yr}^{-1}$ (95% confidence). The error bars represent the range of calculated B_{PCE} using $\epsilon_{\text{PCE}} = -3.3 \pm 1.2\text{‰}$.

To calculate the rate of PCE reduction to TCE, residence times of CEs in the aquifer were derived from measured groundwater residence times (τ_{gw}) that generally increased from south to north and from west to east (Tables 1 and S1). Differences in τ_{gw} (with respect to the source zone) were up to 25 years and average groundwater velocities of 1.5–1.8 m/yr were calculated for the investigated aquifer. Distinct vertical gradients in τ_{gw} reflected the heterogeneity of the aquifer, as can be observed at e.g. location KB5, where the wells KB5a and KB5b tap water from 6–9m ($\tau_{\text{gw}} = 23$ yr) and 10–13m ($\tau_{\text{gw}} = 41$ yr), respectively. This vertical heterogeneity is likely due to the scattered gravel lenses observed in sediment cores, leading to the observed non-uniform spreading of the plume (Figure 1A). Since τ_{gw} as well as isotopic signatures were concurrently measured in every well, this did not hamper the *in-situ* determination of degradation rates.

Accordingly, pseudo-first-order rate constants of PCE transformation were calculated from B_{PCE} -values and the corresponding groundwater residence times using eq 9. Progressive degradation was observed with increasing PCE residence times (Figure 2B). Based on the wells with significant

biodegradation ($B_{PCE} > 20\%$), a pseudo-first-order degradation rate constant k_{PCE} of $0.27 \pm 0.09 \text{ yr}^{-1}$ was calculated, corresponding to half-lives of $2.8 \pm 0.8 \text{ yr}$. Only one well showing a significantly higher degree of dechlorination was excluded from this calculation (well P17, square marker in Figure 2B).

Table 1. Groundwater residence times (τ_{gw}), concentrations, carbon isotopic signatures ($\delta^{13}\text{C}$), and degrees of degradation of PCE and its transformation products, as well as the sum of chlorinated ethenes (CEs) along the transect depicted in Figure 1. See Table S1 in the SI for the comprehensive results of all 37 investigated wells.

well	screen		PCE		TCE		cDCE		VC		$\Sigma(\text{CEs})$		degree of degradation	
label	depth	τ_{gw}	conc	$\delta^{13}\text{C}$	conc	$\delta^{13}\text{C}$	conc	$\delta^{13}\text{C}$	conc	$\delta^{13}\text{C}$	conc	$\delta^{13}\text{C}_{\Sigma\text{CEs}}$	B_{PCE}	$\text{B}_{\Sigma(\text{CEs})}$
	[m]	[yr]	[$\mu\text{mol/L}$]	[‰]	[$\mu\text{mol/L}$]	[‰]	[$\mu\text{mol/L}$]	[‰]	[$\mu\text{mol/L}$]	[‰]	[$\mu\text{mol/L}$]	[‰]	[%]	[%]
RB1/99 ^{a)}	3–9	15.9	279	-23.3	150	-25.7	119	-31.4	19.8	-36.1	568	-26.1	<20 ^{a)}	<5 ^{a)}
P4b	7–8	n.d. ^{b)}	69.1	-16.6	96.4	-27	92.3	-30	12.1	-31.2	270	-25.6	85.9±11.7	<5 ^{c)}
KB2	9–11	18.9	7.41	-18.8	11.4	-22.5	27	-20.5	7.58	-23.5	53.4	-21.1	74.8±18.3	17.0±12.0
P13b	8.5–9.5	36.4	0.145	4.6	2.82	-10.5	58.8	-21.5	4.68	-39	66.4	-22.2	99.9±0.1	14.1±11.2
KB3b	10.5–12.5	13.3	0.108	-4.5	0.992	-9.3	56.3	-24.6	2.26	-34.5	59.6	-24.7	99.2±0.7	<5 ^{c)}
KB6b	10–12	25	<0.05		<0.05		3.09	-6.8	10.5	-26.2	13.6	-21.8	100 ^{d)}	15.3±11.5
P16	7.5–9	n.d. ^{b)}	<0.05		<0.05		<0.05		<0.05		<0.05			

a) Source zone. b) n.d. = not determined since low hydraulic conductivity hampered sampling for groundwater dating. c) No significant degradation resulted from the applied range of ϵ -value and uncertainty in $\delta^{13}\text{C}$ -values. d) 100% degradation, only transformation products of PCE detectable.

The *in-situ* PCE transformation rate constant resulting from this novel isotope-based approach is at the lower end of the range of PCE degradation data (0.15 to 3.7 yr⁻¹) determined at other field situations from concentration measurements combined with artificial tracer tests and/or groundwater modeling (2, 27, 29-31). The presented method thus enables to assess a field site under natural flow conditions and covers time scales much larger (i.e., decades) than the ones normally accessible with artificial tracer experiments (i.e., days to months). In previous studies, investigations therefore mainly focused on bio-stimulated or engineered field sites, which generally exhibit faster groundwater flow as well as higher microbial activities.

Isotopic Mass Balance to Assess the Accumulation of Vinyl Chloride. Due to its high toxicity, VC accumulation is of public health concern at sites with PCE dechlorination (32). Therefore, the assessment of CE biodegradation combined with contaminant residence times in the aquifer was extended to assess the potential of VC accumulation at the field site. For this purpose, isotopic mass balances based on the concentration-weighted ¹³C signatures of the chlorinated ethenes ($\delta^{13}C_{\Sigma(CEs)}$, eq 5) were established, whereas the comparison of $B_{\Sigma(CEs)}$ and the averaged CE residence times allowed to estimate rates of complete PCE transformation to non-toxic (non-chlorinated) products.

Calculated $\delta^{13}C_{\Sigma(CEs)}$ values are presented in Table 1 and S1. As depicted in Figure 3A, $\delta^{13}C_{\Sigma(CEs)}$ -values were within the source value of -26.1±1.2‰ in 9 of 28 wells. This observation implies that CE transformation beyond VC was not significant in these 9 wells, even at residence times of decades. Yet, the shifts in $\delta^{13}C_{\Sigma(CEs)}$ of up to +25‰ observed in the other wells clearly indicate CE dechlorination beyond VC. The degree of the four-step complete PCE dechlorination, $B_{S(CEs)}$, was quantified using the Rayleigh approach according to eq 6 (Tables 1 and S1). The range of corresponding enrichment factors, expressed as $\epsilon_{\Sigma(CEs)}^{\min}$ and $\epsilon_{\Sigma(CEs)}^{\max}$, were calculated with eqs 7-8. We determined a field-specific e-value of -17±3‰ for the sequential PCE-to-VC dechlorination, using a dilution-corrected Rayleigh evaluation in analogy to eq 2 (Figure S2 in SI), and the range of isotopic fractionation factors published for the VC dechlorination ($\epsilon_{VC} = -22\text{‰}$ to -31‰ ; see Table S3). This resulted in $\epsilon_{\Sigma(CEs)}^{\min}$ and $\epsilon_{\Sigma(CEs)}^{\max}$ values of -22‰

and -51‰, respectively. The combined effect of the analytical error of the isotopic mass balance ($\pm 1.2\text{‰}$, obtained by error propagation in eq 5) and the range of $\epsilon_{\Sigma(\text{CEs})}$ values (-22 to -51‰) leads to an absolute quantification limit in $B_{\Sigma(\text{CEs})}$ of 5%. The relative uncertainties of $B_{\Sigma(\text{CEs})}$ were between ± 10 –18%.

Hence, $B_{\Sigma(\text{CEs})}$ -values $>5\%$ are an evidence for on-going transformation of PCE to non-chlorinated products (e.g., ethene). Figure 3A illustrates that shifts in $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$, and thus dechlorination beyond VC, was not occurring at source zone, i.e., at high CE concentrations ($>80 \mu\text{mol L}^{-1}$). However, the $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ -values increased sharply with decreasing CE concentrations at the fringe of the plume, and the corresponding $B_{\Sigma(\text{CEs})}$ -values reached a maximum of $55 \pm 18\%$ at the CE concentration level $<1 \mu\text{mol L}^{-1}$.

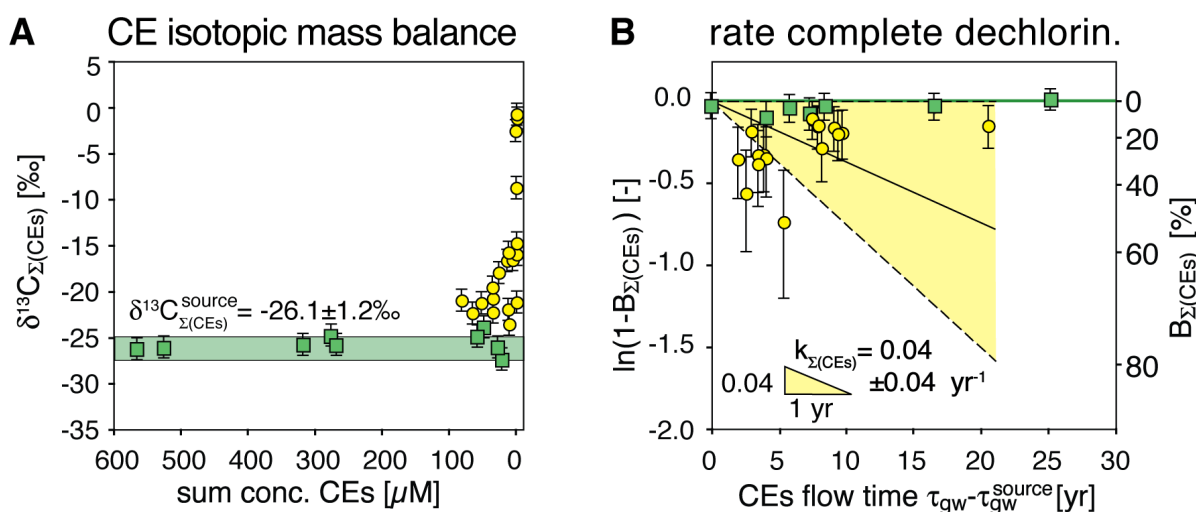


Figure 3. **A)** Isotopic mass balance ($\delta^{13}\text{C}_{\Sigma(\text{CEs})}$) indicating degrees of CE dechlorination to ethene (see text). Error bars represent the analytical uncertainties in concentrations and isotopic signatures. Wells with identical isotopic mass balances as the source zone ($\delta^{13}\text{C}_{\Sigma(\text{CEs})} = -26.1 \pm 1.2\text{‰}$, horizontal bar) are represented by squares. The other wells are represented by circles. **B)** First order degradation rate constants $k_{\Sigma(\text{CEs})}$ of complete dechlorination using $B_{\Sigma(\text{CEs})}$. Wells $B_{\Sigma(\text{CEs})} < 5\%$ are represented by squares. For wells with isotopic mass balance differing from the source zone ($B_{\Sigma(\text{CEs})} > 5\%$; circles), first-order rate constants of $k_{\Sigma(\text{CEs})} = 0.04 \pm 0.04 \text{ yr}^{-1}$ (95% confidence interval, yellow shaded area delimited by dashed lines) resulted for the complete dechlorination of PCE to ethene. Uncertainties were derived from the applied range of $\epsilon_{\Sigma(\text{CEs})}$ -values (-22 to -51‰) and the uncertainty in $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ ($\pm 1.2\text{‰}$). Note that groundwater resident times τ_{gw} were not determined for all wells.

In conclusion, the isotopic mass balance is a strong indication where along the contaminant plume and to what extent VC has a potential to accumulate. Although microbial enrichment cultures, prepared from field sediments, were able to degrade CEs (see Figure S3 in SI) and contained bacteria of the *Dehalococcoides* genus (16S rRNA analysis; P. Rossi, EPFL, Switzerland; personal communication), dechlorination of cDCE and VC happens to be sluggish under the conditions in the contaminated aquifer (see below). This leads to considerable accumulation of VC.

In principle, VC might also be oxidized by anaerobic oxidation or in microaerophilic or hypoxic pockets in the aquifer (13). Although we cannot *a priori* exclude this process, the observed sulfate-reducing conditions in the aquifer as well as the detection of ethene in 9 of a subset of 12 samples (1–28 $\mu\text{g L}^{-1}$) strongly suggest that VC is (slowly) dehalorespired rather than oxidized. Note that our isotope-based approach is still applicable for aquifers with VC oxidation (albeit using different $e_{S(\text{CEs})}$ -values), as VC oxidation is also accompanied by characteristic isotopic shifts (14).

Time Scale of Complete CE Dechlorination. Combining the $B_{\Sigma(\text{CEs})}$ -values with groundwater residence times allowed estimating the rate constants of complete PCE to ethene dechlorination according to eq 10 (Figure 3B). A rate constant of $0.04 \pm 0.04 \text{ yr}^{-1}$ (95% confidence) was calculated from the wells showing ongoing natural attenuation ($B_{\Sigma(\text{CEs})} > 5\%$, circles in Figure 3B). These low rates imply that a remediation strategy based merely on monitoring natural attenuation is not a feasible option, given the time scale of decades to centuries needed for a complete clean up.

However, there are also zones with faster PCE-to-ethene dechlorination. Considering only the four wells with $B_{\Sigma(\text{CEs})} > 30\%$ (wells P10, P13a, P15, B2), the rate constant was $0.18 \pm 0.13 \text{ yr}^{-1}$, corresponding to relatively short half-lives of $8.5 \pm 6.5 \text{ yr}$. This result stipulates the existence of zones with favorable conditions for the *Dehalococcoides spp.* that dechlorinate CEs completely. The scattering of these wells implies that these zones are locally restricted, while limitations of electron-donors (i.e. H_2) might prevail in the rest of the aquifer. An evidence for such limitations is the fact that, e.g., wells with $B_{\Sigma(\text{CEs})}$ -values $> 30\%$ had lower SO_4^{2-} concentration ($\leq 26 \mu\text{mol L}^{-1}$) than other wells (up to $280 \mu\text{mol L}^{-1}$; see Table S2). As recently suggested, *Dehalococcoides spp.* can be out-competed as long as sulfate-reducing

bacteria are active (33), as has also been observed for methanogenic (34) and partly dechlorinating bacteria (35).

Implications. Since the accumulation of toxic VC and the long time scales involved in CE dechlorination are critical at CE contaminated field sites, it is necessary to go beyond qualitative identification of natural attenuation. Using the presented method based on isotope mass balances allowed to quantify the degree of complete PCE dechlorination. Furthermore, the mean groundwater age enabled to estimate the time scale of the contaminant degradation in the assessed confined aquifer. Note, however, that additional groundwater components might need to be considered in unconfined aquifers where inflow of uncontaminated groundwater may not be negligible. The time scale of degradation will therefore need to be disentangled from mean the groundwater age by assessing the effect of the uncontaminated groundwater components on the measured groundwater age.

An important source of uncertainties in calculated degradation rates is the span of employed enrichment factors (ϵ_{PCE} and $\epsilon_{\Sigma(CEs)}$). More research to identify enrichment factors *in-situ* with increased certainty, such as combined carbon and chlorine CSIA (14, 36), is necessary to narrow down the range of field site-specific ϵ -values. Yet, following the presented novel approach allows for quantification of *in-situ* degradation rates and thus substantially contributes to the assessment of contaminated environments. Recent improvements allowing the determination of $\delta^{13}C$ signatures of CEs at ultra-trace concentrations ($<0.5 \mu\text{g/L}$) will be an additional asset in such evaluations (37).

The presented method of combined CSIA and groundwater dating can be applied to determine *in-situ* degradation rates of any other organic groundwater contaminants, such as e.g., MTBE, gasoline components (BTEX), or nitroaromatic compounds.

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Supporting Information Available

Comprehensive tables listing CE concentrations, isotopic signatures and redox-sensitive parameters of all wells, an overview of reported ϵ -values for CE transformation, figures depicting the plume extensions of the individual CEs, dilution corrected Rayleigh-plots for three dechlorination steps, results from microcosms, as well as derivation of eqs 5 and 7-8 and analytical details are provided in the SI. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

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Brief

Rates of chloroethene degradation in contaminated aquifers can be quantified by linking isotopic enrichment with groundwater residence times.