$^{13}C/^{12}C$ Analysis of Ultra-Trace Amounts of Volatile Organic Contaminants in Groundwater by Vacuum Extraction

Helena I. F. AMARAL[§,#,*], Michael BERG[§,#], Matthias S. BRENNWALD[§], Markus HOFER[§], Rolf KIPFER[§,#,*]

[§] Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland.

[#] Institute of Biogeochemistry and Pollutant Dynamics, ETH Zurich, 8092 Zürich, Switzerland.

[*] Corresponding authors; phone: +41-44-8235530; fax: +41-44-8235210; e-mail (H.I.F.A.) helena.amaral@eawag.ch; e-mail (R.K.) rolf.kipfer@eawag.ch

Abstract

We developed a method for the vacuum extraction (VacEx) of volatile organic compounds (VOCs) from water samples for ultra-trace determinations of carbon isotopic signatures. Our method permits compound-specific stable carbon isotope analysis (CSIA) at VOC concentrations of 0.03–1.34 µg/L. VacEx was developed to extract and pre-concentrate VOCs for subsequent carbon-CSIA by the standard technique purge-and-trap (P&T) coupled to an isotope-ratio mass spectrometer (IRMS). Even without complete extraction, the $\delta^{13}C$ signatures of VOCs determined by VacEx-P&T-IRMS were in good agreement (deviation <1‰) with signatures determined by P&T-IRMS. This indicates that VacEx does not cause isotopic discrimination. Limits of quantification (LOQs) for $\delta^{13}C$ analysis were: 0.03-0.06 µg/L for toluene, *o*-xylene, *m*-*p*-xylene and ethylbenzene, 0.15-0.25 µg/L for *trans*-DCE, *cis*-DCE, TCE, methyl tert-butyl ether (MTBE) and benzene, and 0.30 µg/L for PCE. These are the lowest LOQs reported to date for continuous-flow isotope-ratio determinations using a commercially available and automated system. To our knowledge, analytical protocols adopted from noble gas analysis in water were applied for the first time to determine the isotope composition of organic contaminants. We applied VacEx in a field study to illustrate how the determination of VOC isotopic signatures at very low concentrations opens new avenues in the in-situ assessment of these priority groundwater pollutants.

Introduction

Volatile organic compounds (VOCs) are frequent contaminants in soil and groundwater due to tank leakage and other accidental spills in industrialized and urbanized areas. Common industrial VOCs include chlorinated ethenes (CEs), aromatic hydrocarbons and methyl tert-butyl ether (MTBE), widely used in dry-cleaning and degreasing processes, and as gasoline components, respectively. The source and transformation of organic compounds in groundwater has been assessed by compound-specific stable isotope analyses (CSIA, see for example *1-16)*. Moreover, in situ transformation rates can be determined once the contaminated groundwater residence time is known *(15,16)*.

To date, purge-and-trap (P&T) has been considered to be the most efficient extraction technique to pre-concentrate VOCs from water samples for carbon-CSIA *(2)*. Application of P&T

has been developed and optimised to concentrations in the low µg/L range *(2,17)*. However, P&T enrichment for CSIA of many priority pollutants, for example trichloroethene (TCE) isotopic analysis still requires a concentration of at least 1.4 µg/L *(2)*, a value that Jochmann et al. *(17)* could decrease to 1.2 µg/L by increasing the purged water volume from 25 to 100 mL. Thus, the application of CSIA to determine the VOC isotopic composition in the ng/L range, a concentration level frequently found in groundwaters used for drinking-water supply, was up to now not feasible.

To overcome this constraint, we developed an off-line VOC vacuum extraction method (VacEx) by adapting analytical protocols used routinely in the analysis of trace gases in water, particularly noble gases, sulfur hexafluoride and chlorofluorcarbons *(18,20)*. Such techniques are based on degassing water by mechanical shaking under vacuum conditions combined with cryogenic trapping of the volatile species *(19,20)*. VacEx pre-concentrates the analytes in a stainless-steel trap. Subsequently, the trap is connected to a commercial P&T-gas-chromatograph (GC)-IRMS system for CSIA analysis following the protocols described in Zwank et al. *(2)*. The described VacEx method was validated for the determination of $\delta^{13}C$ signatures at ultra-trace concentration levels of tetrachloroethene (PCE), trichloroethene (TCE), and dichloroethene (DCE, isomers *cis* and *trans*), aromatic hydrocarbons (BTEX; benzene, toluene, ethylbenzene and the xylene isomers), and methyl tert-butyl ether (MTBE). Furthermore, we applied VacEx in a field study to assess the origin of the PCE and TCE found in groundwater used for drinking-water production.

Experimental Section

Reagents and Standard Preparation

The physicochemical properties of the target compounds used are listed in Table S1 of the supporting-information (SI) section. Methanol (>99.9%; Scharlau S.A., Barcelona, Spain) was used to prepare stock solutions. TCE (≥99%), *trans*-1,2-DCE (98%), *cis*-1,2-DCE (97%), and PCE (99%) were obtained from Sigma-Aldrich (Steinheim, Germany). Benzene (≥99.9%), ethylbenzene (≥99.5%), MTBE (≥99.5%), toluene (≥99.9%), *o*-xylene (≥99.5%), *m*-xylene (≥99.5%) and *p*-xylene (≥99.5%) were purchased from Fluka (Buchs, Switzerland).

Laboratory samples for the validation of VacEx and the comparison with P&T were prepared in tap water with target-compound concentrations below the detection limit. To avoid

air entrapment, the 500 mL VacEx sample container was filled from the bottom end in a vertical position and closed after flushing it at least by three times its volume. Likewise, the 25 mL P&T vials were slowly filled to avoid gas bubbles to be formed and closed without headspace. A respective amount of the analyte standard was then added to the VacEx container and the P&T vials to obtain identical VOCs concentrations in both samples.

Two different types of analyte standards were prepared: methanolic stock solutions and aqueous standard solutions (S_0). Three methanolic stock solutions contained 4000 ppmV of each CE, of each BTEX, and MTBE, respectively. These stock solutions were prepared in 25 mL volumetric flasks by spiking 100 µL of the corresponding analytes with gas tight Hamilton glass syringes to the methanol. Subsequently, two aqueous standards were prepared in tap water at different concentration levels. In S_{01}, 100 µL of each of the three methanolic standards were added to 25 mL of water (16 ppmV of each CE, MTBE and BTEX) and in S_{02}, 100 µL of the CE methanolic solutions and 20 µL each of the methanolic solutions of BTEX and of the MTBE were added (16 ppmV of each CE and 3.2 ppmV BTEX and MTBE). S_{02} was used for most of the experiments because S_{01} contained too much BTEX for CSIA.

Groundwater Sampling

At least three times the volume of the entire well volume was pumped from the groundwater wells by means of a submersible pump (MP1, Grundfos) before a sample was taken. Prior to sampling, the VacEx sample containers were cleaned in the laboratory with acetone. The sample container was connected to the hose of the submersible pump and filled bottom up assuring that no bubbles were entrapped. To minimize volatilization of the analytes during sampling and transport to the laboratory, the water samples for P&T were collected in 120 mL glass containers and sealed with PTFE-lined screw caps. The vials were slowly filled and sealed without headspace. All samples were stored in the fridge at 4 °C and without adding any preservatives.

Vacuum Extraction (VacEx)

Figure 1 illustrates the setup of the off-line vacuum extraction. All components are commercially available, except the tailor-made extraction vessel and cold traps. All components are made of stainless steel to avoid adsorption of the organic compounds. Manually operated valves (SS-4P4T, Swagelok) separate the different sections of the system.

The sample container (SS in Figure 1; 304L-HDF4-500, Swagelok) is a 500 mL gas-tight stainless steel vessel with valves V1 and V2 at both ends. Prior to extraction, one side of the sample container is connected to the extraction vessel by a flexible rubber hose (RH; 100 mm long and 10 mm internal diameter). The other side is connected to a 1 m long flexible metal hose (MH; PF 530 010-X DN16, Pfeiffer Vacuum). To avoid condensation of water vapour during VOCs extraction, the bellow is heated to 50 °C using a heating tape. Four capillaries (C; stainless steel, 15 mm long, 0.8 mm inner diameter), mounted between the flexible metal hose and the cold trap T1 limit the vapour flux to the cold traps and prevent back-diffusion of the volatile analytes *(20)*.

The cold traps T1 and T2 are made of 300 mm long steel tubing with inner and outer diameters of 3 and 5 mm, respectively. During extraction both traps are submerged in liquid nitrogen (-196 °C) to freeze out the analytes and the water vapour. The traps are bent to a loop in order to foster turbulent gas flow. The turbulence causes the condensable gases to hit the walls of the cold traps, where the gases are trapped. Traps T1 and T2 are installed in series to assure complete condensation of the target analytes. Trap T3 is attached to a rotary vane pump (RP) and is permanently submerged in liquid nitrogen to avoid back-flush of the pumped gases to the extraction line.

Prior to sample extraction, the line is evacuated in two stages. First, a membrane pump (not shown in Figure 1; MD 1 Vario-SP, Vacuubrand GMBH + CO KG) is used to remove the water vapour from the system (final pressure: ~1 mbar). In a second step, the rotary vane pump is used to achieve a vacuum of <0.05 mbar in the line. The pressure is monitored with a Pirani gauge (P; PRL 10K, Edwards) placed between the traps T2 and T3.

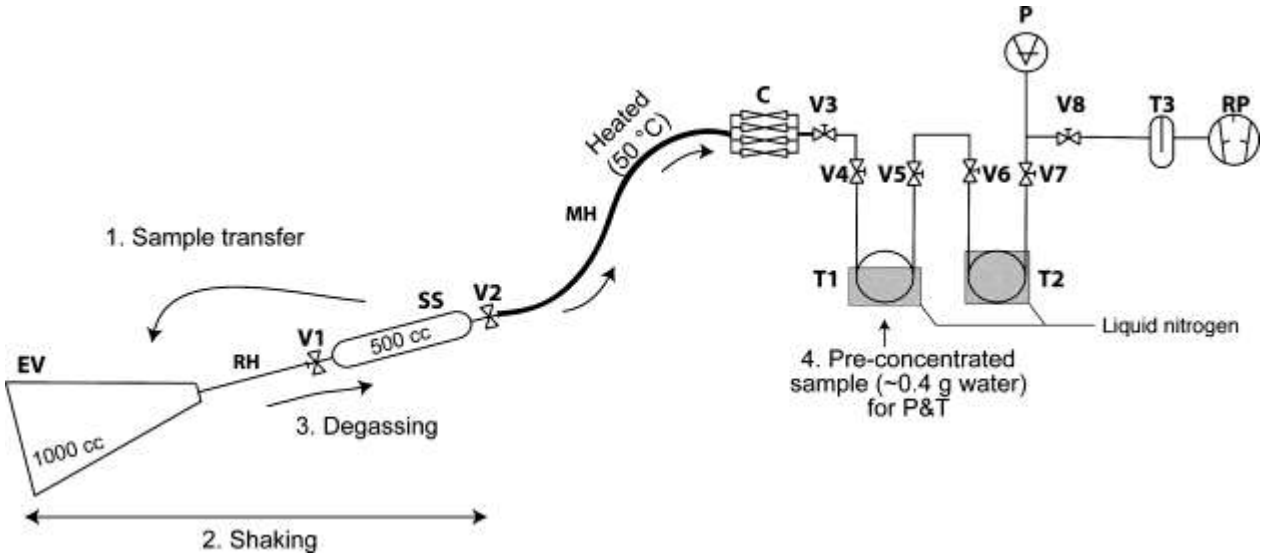

Figure 1. Diagram of the vacuum extraction line (VacEx), depicting its components and the major steps in the extraction of VOCs from water samples. The procedure is described in the text.

Before gas extraction, all valves are open except valves V1 and V2, and the traps T1 and T2 are cooled by liquid nitrogen. In a first step, the water in the sample container is transferred to the extraction vessel by opening valve V1, while valve V2 remains closed. In a second step, the sample is degassed by opening V2 and shaking both the extraction vessel and sample container at 120 rotations-per-minute using a mechanical shaker. All valves of the extraction line are open. As the water degasses, the pressure difference in the line obtained by vacuum causes the water vapour and the extracted gases to be transferred (and freezed) to the traps T1 and T2, after being forced to pass the capillary. The small diameter of the capillary causes the gas velocity to increase strongly, which prevents back diffusion of the extracted gases *(20)*. To avoid water ice clogging that might disrupt the gas flow, the loop of trap T1 is only half-ways dipped into liquid nitrogen, whereas the loop of trap T2 is completely submerged. If both cold traps were completely submerged up to the valves V4-V7, the extraction efficiency was considerably reduced (up to 50 %) due to clogging by water ice.

As VOCs condense at temperatures significantly higher than that of liquid nitrogen (Table S1 in the SI section), they are quantitatively trapped together with water vapour in trap T1 (and to a very small degree in trap T2). In contrast, the atmospheric gases N_2, O_2, and Ar remain in the gas phase, and are thus continuously transferred to the rotary vane pump, thereby maintaining a steady gas flow *(18)*. Several tests showed that VOCs were to a large extent

retained in trap T1, with less than 3% thereof retained in trap T2. Based on this robust and reproducible observation, trap T2 is not entirely essential for the extraction method. In the final step, valves V4 and V5 are closed under vacuum, trap T1 is brought to room temperature and detached from the system, while valves V3 and V6 are kept closed to prevent the sudden inflow of air to the system. Valves V3 and V8 act as safety valves used when a new sample is attached to the extraction line and the whole system is pumped before a new extraction. From a water sample of 500 mL, about 0.4 mL of water are retained in trap T1 during an extraction lasting 40 min. The VOCs extracted are concentrated in these 0.4 mL of water.

Isotopic Analysis of VOCs Concentrated by VacEx

We analysed the aqueous concentrations and the $\delta^{13}C$ signatures of the VOCs. VacEx basically transfers and pre-concentrates all VOCs in trap T1 for further P&T-GC-IRMS analysis. Because VacEx and standard P&T are hypothesized to yield the same results, we have directly compared the results obtained from both methods. The highest evaluated VOC concentrations were chosen with the intention to obtain maximum IRMS signal amplitudes of ~10 V (m/z = 44). This assured isotopic measurements in the linear range of the IRMS (0–12 V).

The VOCs extracted by VacEx were measured for isotopic determination by P&T-GC-IRMS (details in reference 2). Briefly, trap T1 containing the pre-concentrated sample was connected to the P&T system (Tekmar LSC3100, Tekmar-Dohrmann). To attach the trap to the P&T system, the manufacturers P&T glass-vial was removed and trap T1 was attached instead. One end of trap T1 was connected to the input of the trap of the P&T system using a stainless steel tube. The other end of trap T1 was connected to a hose of the purging system. The P&T trap was coupled to the pre-column of the GC-IRMS system via a cryo-focusing unit. After activating the N_2 purging of the P&T system, valves V1 and V2 were opened simultaneously, allowing the purge gas to circulate through trap T1, which was purged for 11 minutes at a N_2 flow rate of 40 mL/min. The analytes purged from trap T1 were trapped on the P&T trap (Supelco, VOCARB 300) at room temperature. Subsequently, the analytes were thermo-desorbed from the P&T trap by heating it to 250 °C and then carried by pure helium to the cryo-focusing unit maintained at -120 °C using liquid nitrogen. Finally, the analysis on the GC (Trace GC, Thermo Finnigan) was

finally started simultaneously with the heating of the cryo-focusing unit, whereby the analytes were transferred by the He carrier gas to the GC column for chromatographic separation.

The compound-specific isotope ratios were determined using an IRMS (Delta^{PLUS}XL, Thermo Finnigan MAT) via a combustion interface (GC Combustion III, Thermo Finnigan MAT) operating at 940°C. The GC was equipped with a deactivated pre-column (0.5 m x 0.53 mm, BGB) and a Restek RTX-VMS capillary column (60 m x 0.32 mm, 1.8 µm film thickness, Restek Corp.). The carrier-gas pressure was kept constant 100 kPa. The GC temperature program was as follows: 2 min at 40 °C, then to 50 °C at 2 °C/min, 4 min at 50 °C, then to 100 °C at 8 °C/min, 2 min at 100 °C, then to 210 °C at 40 °C/min, 3.5 min at 210 °C. In addition to the IRMS, the GC was equipped with a flame ionisation detector (FID) that received approximately 10% of the gas flow from the column.

The IRMS provides the relative abundances of ^{13}C and ^{12}C, expressed by the ratio $^{13}C/^{12}C$. The bulk isotope ratios are commonly reported in the notation with respect to the Vienna PeeDee Belemnite international standard (VPDB, *1)* with $(^{13}C/^{12}C)_{VPDB} = 0.011237$:

$$\delta^{13}C = \frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{VPDB}} - 1$$

Determination of Extraction Efficiencies and Limits of Quantification

The effect of extraction efficiencies on the reproducibility of isotopic measurements was evaluated by (a) variation of VOC extraction times, and (b) variation of VOC concentrations. VacEx extraction efficiencies were determined from the FID signal of the GC by comparing the analyte peak areas obtained from standards concentrated by VacEx with those obtained from P&T.

The Limit of quantification (LOQ) for isotope-ratio analysis with VacEx was determined using two approaches:

The LOQ corresponds to the lowest concentration of a target analyte at which the deviation between the ^{13}C value measured using VacEx relative to that measured by standard P&T was <1 ‰.

- The LOQ corresponds to the concentration which generated an IRMS amplitude of 0.5 V (*m/z* = 44). This is in agreement with the technical specifications of the IRMS to obtain reliable isotope signatures.

The stated reproducibility corresponds to the standard deviation of three (VacEx) to six (P&T) replicate measurements.

Results and Discussion

Extraction Efficiencies

a) Variation of Extraction Time. Samples were extracted during 10, 20, 30, 40 and 50 minutes in triplicate experiments. The VOC concentrations (prepared from standard S_{01}) were: 2.13 µg/L *c*-DCE, 2.08 µg/L *t*-DCE, 2.42 µg/L TCE, 2.7 µg/L PCE, 1.23 µg/L MTBE, 1.46 µg/L benzene, 1.44 µg/L toluene, 1.44 µg/L ethylbenzene, 2.86 µg/L m-p-xylene and 1.43 µg/L o-xylene. The extraction efficiencies generally ranged between 50-110%, but were occasionally higher for MTBE and somewhat lower for ethylbenzene and xylenes (30-50%). Figure 2 shows typical results obtained for PCE, TCE, MTBE and c-DCE. No trend in efficiency was observed with increasing extraction times, yet, the amount of vacuum-extracted analytes varied considerably between triplicate experiments with average standard deviations of ±20%. As mentioned in the section 'Vacuum Extraction (VacEx)', this variability was partly related to water ice clogging in trap T1, because this trap was fully submerged in liquid nitrogen during initial tests of the method. Keeping the loop of trap T1 only halfway dipped into liquid nitrogen increased the reproducibility considerably.

However, the variability in the extraction efficiencies did not affect the precision of the $\delta^{13}C$ values. Independently of the VOC extraction time, the isotopic compositions obtained from VacEx and P&T differed by <0.6‰ for all compounds, except for *t*-DCE (1.2‰) and of TCE (1‰, Table S2 in the SI section). This indicates that VacEx did not interfere with the isotopic signature of the target compound. We did not observe any systematic trend of C-isotope fractionation as a function of extraction time.

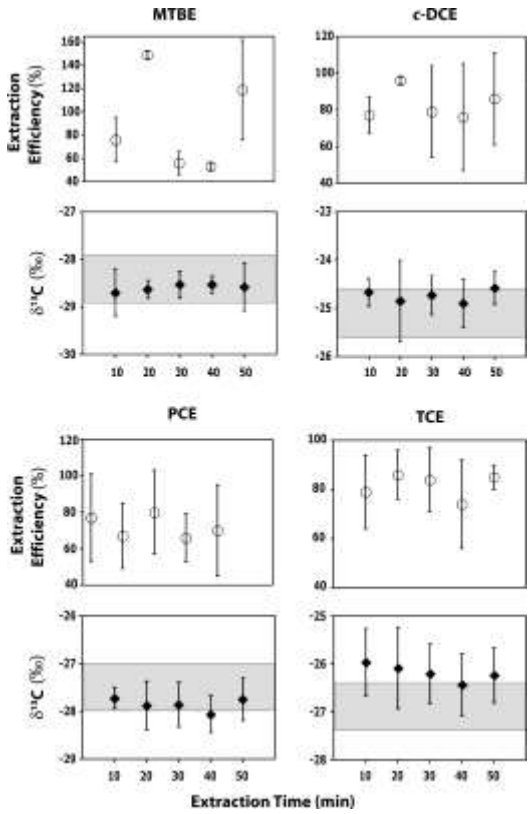

Figure 2. Influence of extraction times (10–50 min) on the extraction efficiency (open circles; note different scaling) of selected analytes and their isotopic compositions (black diamonds). The gray bar corresponds to isotopic signature ($\delta^{13}C \pm 0.5‰$) of the analytes determined by P&T-IRMS. Error bars indicate the standard deviation (1σ) of triplicate VacEx experiments.

b) Variation of VOC Concentrations. Triplicates of samples with varying VOC concentrations were prepared from standard S_{02} and extracted for 40 minutes. The range of VOC concentrations were: 0.21-4.25 µg/L *c*-DCE, 0.21-4.16 µg/L *t*-DCE, 0.24-4.84 µg/L TCE, 0.27-5.38 µg/L PCE, 0.02-0.49 µg/L MTBE, 0.03-0.58 µg/L benzene, 0.03-0.57 µg/L toluene, 0.03-0.57 µg/L ethylbenzene, 0.06-1.16 µg/L *m*-*p*-xylene and 0.03-0.58 µg/L *o*-xylene. The highest VOC concentrations resulted in IRMS peak amplitudes below 12 V and were thus in the linear range of $\delta^{13}C$ determinations (see Experimental Section). Figure 3 shows the $\delta^{13}C$ values obtained for *c*-DCE, *t*-DCE, TCE and toluene. The extraction efficiencies ranged between 80-130%, except for *o*-Xylene (55-90%), and were found to be virtually independent of the VOC concentrations. Again, large standard deviations (>40%) were found to be decoupled from the VOC concentrations.

The $\delta^{13}C$ of all VacEx measurements agree with those determined with the standard P&T method. A minor increase in the TCE $\delta^{13}C$ signature of ~1‰ or less throughout the range of the tested analyte concentrations cannot be ruled out (Figure 3). In general, however, we did not observe any systematic trend of C-isotope fractionation as a function of analyte concentration.

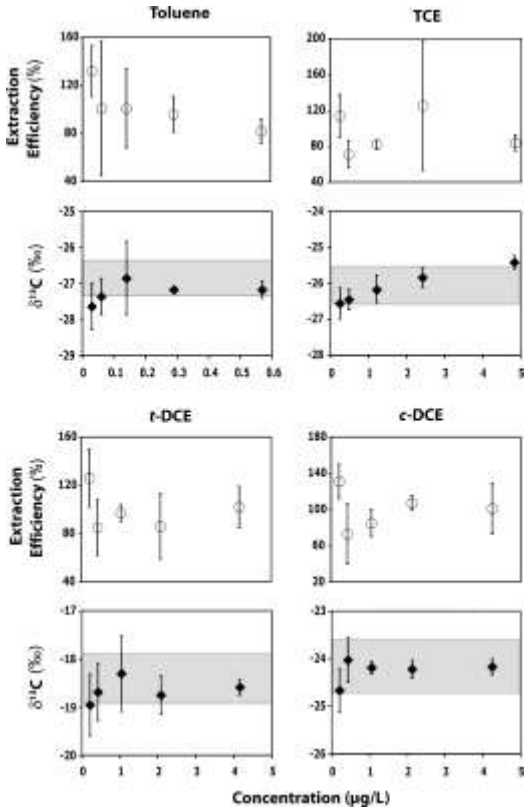

Figure 3. Influence of analyte concentrations (0.03–4.8 µg/L) on the extraction efficiency (open circles; note different scaling) and their isotopic compositions (black diamonds). The gray bar corresponds to the isotopic signature ($\delta^{13}C \pm 0.5$‰) of the analyte determined by P&T-IRMS. Error bars indicate the standard deviation (1σ) of triplicate VacEx experiments.

Accuracy and Precision of VacEx $\delta^{13}C$ Determination and Validation with P&T

The carbon isotope signatures of the analytes pre-concentrated by VacEx deviated <0.6‰ from P&T. The typical error of the isotopic measurement was in the range of ±0.5–1‰. Hence, VacEx-P&T-IRMS produced accurate $\delta^{13}C$ measurements at the sub-microgram concentration level with standard deviations <1‰, independent of extraction time or analyte concentration levels. $\delta^{13}C$ deviations > 1‰ (see Table 1 and Table S3 in SI section) were observed at the

lowest tested concentrations of benzene (2.17‰ at 0.03 µg/L), PCE (1.65‰ at 0.27 µg/L), ethylbenzene (1.22‰ at 0.03 µg/L) and MTBE (1.71‰ at 0.02 µg/L).

The LOQs of VacEx achieved for $\delta^{13}C$ measurements by CSIA are listed in Table 1. The developed off-line analyte pre-concentration by VacEx lowered the LOQs of carbon-CSIA by P&T by factors ranging from ~2 to ~8 (Table 1), i.e. to a VOC concentrations of 0.03 µg/L to 1.34 µg/L. Figure 4 illustrates the agreement of the $\delta^{13}C$ signatures measured using VacEx with those measured using P&T at the LOQs. PCE is the compound showing the highest LOQ in both the VacEx (0.30–1.3 µg/L) and P&T (2.2 µg/L) methods (see Table 1). Although PCE is the compound with the lowest solubility in water (thus expected to be the easiest to extract from water) and the highest boiling point (thus expected to be the easiest to be retained in the cold traps), it is the compound for which VacEx yields the least improvement. At this point it is likely that the comparatively high LOQ for PCE is related to the extraction method rather than the IRMS analysis. Thus, if a further decrease of the LOQ is desired, the focus should be on the improvement of the extraction method. With the possible exception of PCE, the LOQs do not seem to be associated with the IRMS sensitivity, because the amount of analytes extracted was enough for CSIA by VacEx. In principle, the VacEx LOQs can be lowered by extracting even larger water samples (>0.5 L).

TABLE 1. Limits of quantification (LOQ) for the $\delta^{13}C$ determination of organic groundwater contaminants by VacEx and P&T.

Compound	**LOQ** (µg/L in water)		
	P&T[a]	**VacEx**	
		accuracy ±1‰[b]	amplitude 0.5 V[c]
t-DCE	1.5	0.21	0.23
c-DCE	1.1	0.21	0.18
TCE	1.4	0.24	0.24
PCE	2.2	1.34	0.30 (-1.7‰)
MTBE	0.63	0.25	0.09 (+1.7‰)
Benzene	0.3	0.15	0.03 (-2.2‰)
Toluene	0.25	0.03	0.03 (-1.2‰)
Ethylbenzene		0.06	0.04
mp-Xylene		0.06	0.06
o-Xylene		0.03	0.05

[a] According to Zwank et al. *(2)*. [b] Corresponding to <1‰ deviation in $\delta^{13}C$ values relative to P&T. [c] Corresponding to an IRMS peak amplitude of 0.5 V ($\delta^{13}C$ deviations >1‰ are indicated in brackets).

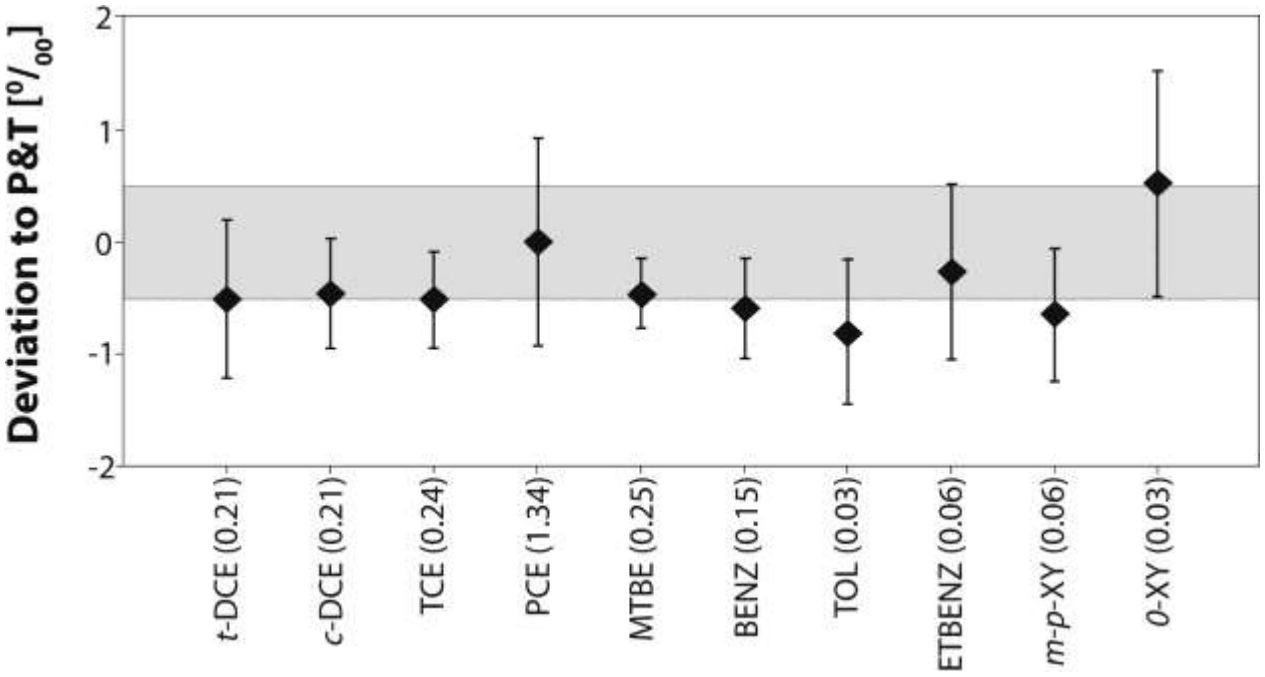

Figure 4. Deviation of the $\delta^{13}C$ of the compounds determined by VacEx relative to the $\delta^{13}C$ determined by P&T. The dark grey bar represents the typical error of the isotopic composition as measured by P&T (±0.5‰). The minimum concentrations (in µg/L) for which the $\delta^{13}C$ deviation between both methods was <1‰ is shown in brackets below the x-axis.

Environmental Application of VacEx-CSIA

The applicability of the VacEx method for the determination of $\delta^{13}C$ values at low VOC concentrations was tested with samples from a contaminated field site showing concentrations of PCE and TCE as low as 0.5 µg/L in the groundwater (see also SI section). The objective was to allocate the source of PCE and TCE present in a drinking water producing groundwater well. A known contaminated site in the aquifer about 1 km upstream has been suggested to be the source. However, the origin of PCE and TCE at the pumping station was never verified, because the concentrations were too low for reliable $\delta^{13}C$ determinations. With the presented VacEx method, we were able to demonstrate that TCE and PCE in the pumping station do not originate from the contaminated site in question. The measured $\delta^{13}C$ signatures shown in Figure 5 (and Table S5 in the SI) illustrate that the PCE and TCE at the pumping station were isotopically lighter than at the contaminated site. Since the transformation (i.e., dechlorination) of either PCE or TCE is known

to lead to isotopically heavier composition along the contaminant plume *(23)*, it can be concluded that PCE and TCE at the pumping station do not originate from the suspected site, but from a so far unidentified source of contamination.

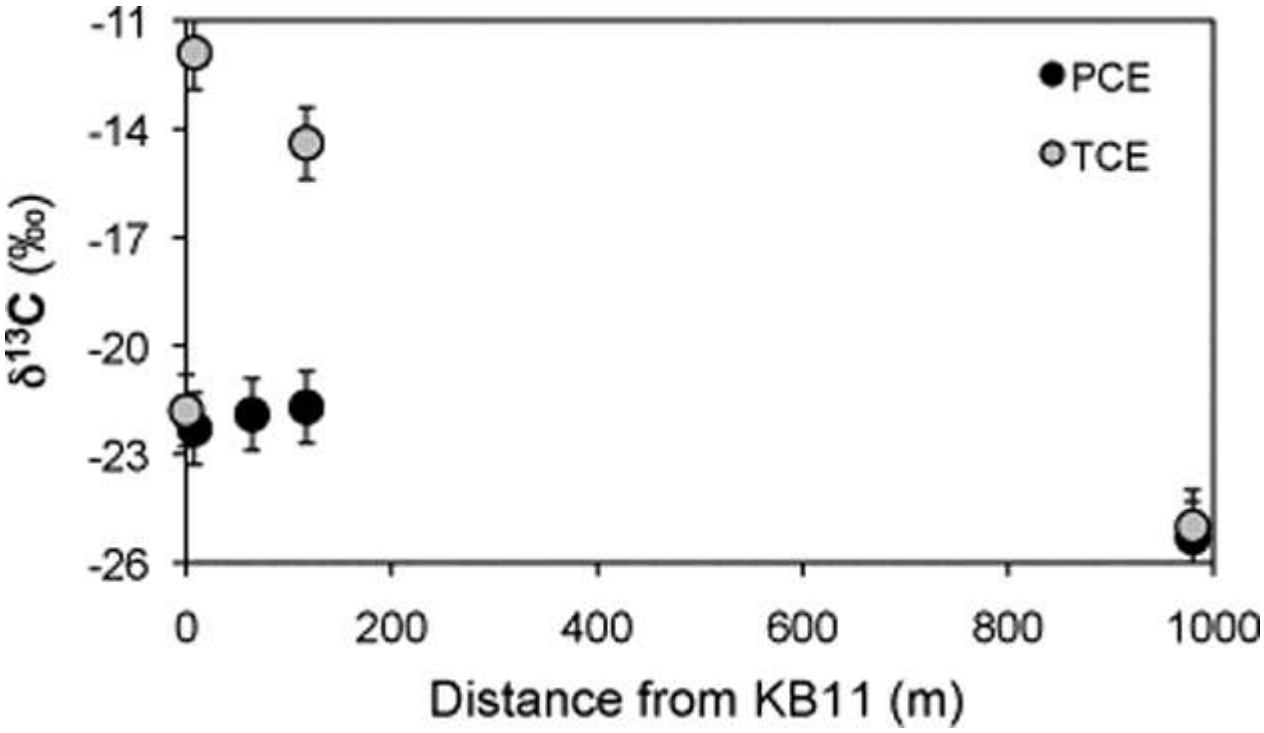

Figure 5. $\delta^{13}C$ evolution of PCE and TCE along the groundwater flow path.

This field study demonstrates that the VacEx development represents a step forward in assessing the origin and the in-situ transformation of VOCs, as well as in evaluating the risk of such compounds to groundwater used for drinking-water supply.

Acknowledgements

The authors are grateful to Christoph Aeppli, Jakov Bolotin, Thomas Hofstetter and Nicole Tobler for their support with GC-IRMS measurements, Jasmin Mertens for initial VacEx trials, and Peter Gäumann for mechanical assistance. The authors thank the three anonymous reviewers for their helpful comments and suggestions on the manuscript.

Supporting Information Available

The supporting-information section contains the physico-chemical properties of the analytes considered, all experimental data, and a more detailed description of the field site used to assess VacEx. This material is available free of charge via the internet at http://pubs.acs.org.

References

(1) Schmidt, T. C.; Zwank, L.; Elsner, M.; Berg, M.; Meckenstock, R. U.; Haderlein, S. B. Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges. *Anal. Bioanal. Chem.* **2004**, *378*, 283-300.

(2) Zwank, L.; Berg, M.; Schmidt, T. C.; Haderlein, S. Compound-Specific Carbon Isotope Analysis of Volatile Organic Compounds in the Low-Microgram per Liter Range. *Anal. Chem.* **2003**, *75*, 5575-5583.

(3) Hunkeler, D.; Butler, B. J.; Aravena, R.; Barker, J. F. Monitoring biodegradation of methyl *tert*-Buthyl Ether (MTBE) using compound-specific carbon isotope analysis. *Environ. Sci. Technol.* **2001**, *3*, 676-681.

(4) Sherwood Lollar, B.; Slater, G. F.; Sleep, B.; Witt, M.; Klecka, G. M.; Harkness, M.; Spivack, J. Stable Carbon Isotope Evidence for Intrinsic Bioremediation of Tetrachloroethene and Trichloroethene at Area 6, Dover Air Force Base. *Environ. Sci. Technol.* **2001**, *35*, 261-269.

(5) Van Stone, N.; Przepiora, A.; Vogan, J.; Lacrampe-Couloume, G.; Powers, B.; Perez, E.; Maury, S.; Sherwood Lollar, B. Monitoring trichloroethene remediation at an iron permeable reactive barrier using stable carbon isotopic analysis. *J. Contam. Hydrol.* **2005**, *78*, 313-325.

(6) Morrill, P. L.; Lacrampe-Couloume, G.; Slater, G. F.; Sleep, B. E.; Edwards, E. A.; McMaster, M. L.; Major, D. W.; Sherwood Lollar, B. Quantifying chlorinated ethene degradation during reductive dechlorination at Kelly AFB using stable carbon isotopes. *J. Contam. Hydrol.* **2005**, *76*, 279-293.

(7) Slater, G. F.; Sherwood Lollar, B.; Sleep, B. E.; Edwards, E. A. Variability in Carbon Isotopic Fractionation during Biodegradation of Chlorinated Ethenes: Implications for Field Applications. *Environ. Sci. Technol.* **2001**, *35*, 901-907.

(8) Bloom, Y.; Aravena, R.; Hunkeler, D.; Edwards, E.; Frape, S. K. Carbon isotope fractionation during microbial dechlorination of trichlorethene, cis-1,2-dichlorethene, and vinyl chloride: implications for assessment of natural attenuation. *Environ. Sci. Technol.* **2000**, *34*, 2768-2772.

(9) Gray, J. R.; Lacrampe-Couloume, G.; Gandhi, D.; Scow, K. M.; Wilson, R. D.; Mackay, D. M.; Sherwood Lollar, B. Carbon and Hydrogen Isotopic Fractionation during Biodegradation of Methyl tert-Butyl Ether. *Environ. Sci. Technol.* **2002**, *36*, 1931-1938.

(10) Cichocka, D.; Imfeld, G.; Richnow, H.-H.; Nijenhuis, I. Variability in microbial carbon isotope fractionation of tetra- and trichloroethene upon reductive dechlorination. *Chemosphere* **2008**, *71*, 639-648.

(11) McKelvie, J. R.; Mackayb, D. M.; Sieyesb, N. R.; Lacrampe-Couloumea, G.; Sherwood Lollar, B.. Quantifying MTBE biodegradation in the Vandenberg Air Force Base ethanol release study using stable carbon isotopes. *J. Contam. Hydrol.* **2007**, *94*, 157-165.

(12) Drenzek, N. J.; Eglinton, T. I.; Wirsen, C. O.; May, H. D.; Wu, Q.; Sowers, K. R.; Reddy, C. M. The Absence and Application of Stable Carbon Isotopic Fractionation during the Reductive Dechlorination of Polychlorinated Biphenyls. *Environ. Sci. Technol.* **2001**, *35*, 3310-3313.

(13) Bernstein, A.; Ronen, Z.; Adar, E.; Nativ, R.; Lowag, H.; Stichler, W.; Meckenstock, R. U. Compound-Specific Isotope Analysis of RDX and Stable Isotope Fractionation during Aerobic and Anaerobic Biodegradation. *Environ. Sci. Technol.* **2008**.

(14) Amaral, H. I. F.; Fernandes, J.; Berg, M.; Schwarzenbach, R. P.; Kipfer, R. Assessing TNT and DNT groundwater contamination by compound-specific isotope analysis and ^{3}H–^{3}He groundwater dating: A case study in Portugal. Chemosphere **2009**, *77*, 805-812.

(15) Aeppli, C.; Hofstetter, T. B.; Amaral, H. I. F.; Schwarzenbach, R. P.; Berg, M. Quantifying in-situ Transformation Rates of Chlorinated Ethenes: Novel Approach Combining Compound-Specific Stable Isotope Analysis and Groundwater Dating. *submitted* to *Environ. Sci. Technol.* **2009**.

(16) Amaral, H. I. F.; Aeppli, C.; Berg, M.; Schwarzenbach, R. P.; Kipfer, R. Combination of Compound-specific Stable Isotope Analysis and Environmental Tracers to Assess in situ Natural Transformation of Chlorinated Ethenes in Groundwater. In revision for Journal of Contaminant Hydrology, **2009**.

(17) Jochmann, M. A.; Blessing, M.; Haderlein, S. B.; Schmidt, T. C. A new approach to determine method detection limits for compound-specific isotope analysis of volatile organic compounds. *Rapid Commun. Mass Sp.* **2006**, *20*, 3639-3648.

(18) Hofer, M.; Imboden, D. M. Simultaneous determination of CFC-11, CFC-12, N_2 and Ar in water. *Anal. Chem.* **1998**, *70*, 724-729.

(19) Kipfer, R.; Aeschbach-Hertig, W.; Peeters, F.; Stute, M. In *Noble gases in geochemistry and cosmochemistry*; Porcelli, D., Ballentine, C. J., Wieler, R., Eds.; Mineralogical Society of America, Geochemical Society, 2002; Vol. 47, pp 615-700.

(20) Beyerle, U.; Aeschbach-Hertig, W.; Imboden, D. M.; Baur, H.; Graf, T.; R., K. A Mass Spectrometric System for the Analysis of Noble Gases and Tritium from Water Samples. *Environ. Sci. Technol.* **2000**, *34*, 2042-2050.

(21) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. In *Environmental Organic Chemistry*, 2003.

(22) Linde, D. R. *CRC Handbook of Chemistry and Physics*; 87th ed.; CRC Press: Boca Raton, FL, 2006.

(23) Hunkeler, D.; Aravena, R.; Butler, B. L. Monitoring Microbial Dechlorination of Tetrachloroethene (PCE) in Groundwater Using Compound-Specific Stable Carbon Isotope Ratios: Microcosm and Field Studies. *Environ. Sci. Technol.* **1999**, *33*, 2733-2738.

Table of Contents Brief:

A new method for compound-specific $\delta^{13}C$ analysis of volatile organic compounds at unprecedentedly low concentrations in groundwater.