

Demonstrating a Natural Origin of Chloroform in Groundwater Using Stable Carbon Isotopes

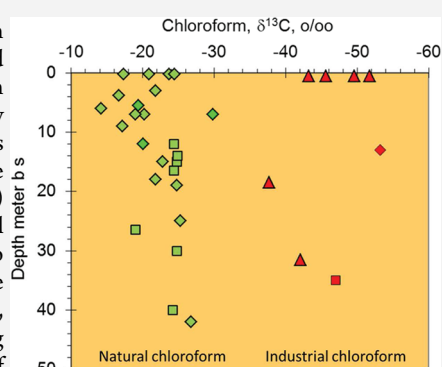
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Supporting Information

ABSTRACT: Chloroform has been for a long time considered only as an anthropogenic contaminant. The presence of chloroform in forest soil and groundwater has been widely demonstrated. The frequent detection of chloroform in groundwater in absence of other contaminants suggests that chloroform is likely produced naturally. Compound-specific isotope analysis of chloroform was performed on soil-gas and groundwater samples to elucidate whether its source is natural or anthropogenic. The $\delta^{13}\text{C}$ values of chloroform (-22.8 to -26.2‰) present in soil gas collected in a forested area are within the same range as the soil organic matter (-22.6 to -28.2‰) but are more enriched in ^{13}C compared to industrial chloroform (-43.2 to -63.6‰). The $\delta^{13}\text{C}$ values of chloroform at the water table (-22.0‰) corresponded well to the $\delta^{13}\text{C}$ of soil gas chloroform, demonstrating that the isotope signature of chloroform is maintained during transport through the unsaturated zone. Generally, the isotope signature of chloroform is conserved also during longer range transport in the aquifer. These $\delta^{13}\text{C}$ data support the hypothesis that chloroform is naturally formed in some forest soils. These results may be particularly relevant for authorities' regulation of chloroform which in the case of Denmark was very strict for groundwater ($<1\ \mu\text{g/L}$).



INTRODUCTION

Chlorinated hydrocarbons are among the most frequently detected contaminants in groundwater worldwide. For example in the U.S., chloroform (CHCl_3) was detected ($>0.2\ \mu\text{g/L}$) in 26.4% of urban and 5.1% of rural wells, trichloroethene (TCE) in 11.6% of urban and 1.6% of rural wells and tetrachloroethene (PCE) in 16.6% of urban and 2.5% of rural wells.^{1,2} In the Danish national groundwater quality monitoring program, chloroform was detected in 8% of the wells, TCE in 9% and PCE in 6.2%.³ Chlorinated hydrocarbons in groundwater are generally considered to be of anthropogenic origin only. However, it has been demonstrated that chlorinated hydrocarbons can also be naturally produced and more than 3800 naturally occurring halogenated compounds have been identified.⁴ Among the frequently detected chlorinated groundwater contaminants, chloroform has been shown to be produced naturally,⁵ while a widespread natural production of TCE and PCE seems less likely.

Natural chloroform production in forest soils has been demonstrated using spatial and temporal concentration patterns,⁵⁻⁸ laboratory incubation experiments and in situ field incubations with ^{37}Cl .^{9,10} Different pathways have been proposed for natural chloroform production. When incubating organic compounds (organic acids, glucose) with chloroperoxidase and hydrogen peroxide, chloroform was detected as the main reaction product.¹¹ The reaction was postulated to proceed via the formation of reactive chlorine species such as

hypochlorous acid and thus is analogous to the formation of chloroform during chlorination of water.^{9,12} Since chloroperoxidases likely originating from wood-rotting fungi were detected in forest soils,^{13,14} it was postulated that a similar production mechanism also occurs in soils. Another proposed pathway is the formation of chloroform by decarboxylation of trichloroacetic acid that is itself product of chlorination of organic matter in soil or originates from atmospheric oxidation of chlorinated ethenes.⁹ Recently, it was demonstrated that chloroform can also be produced from organic model substance in a Fenton-like process in the absence of chloroperoxidases.¹⁵

For groundwater management and remediation, it is important to know to what extent chloroform in groundwater originates from natural sources. In Denmark which rely heavily ($>95\%$) on clean groundwater for its portable water supply this knowledge proved to be particularly urgent in recent years. A maximum of $1\ \mu\text{g/L}$ of volatile halogenated hydrocarbons including chloroform is allowed for groundwater to be used for drinking water, although up to $25\ \mu\text{g/L}$ is permitted if chlorination is needed for disinfection. The strict regulation for groundwater with respect to chloroform compared to drinking water is due to a general fear of pollution, assuming that

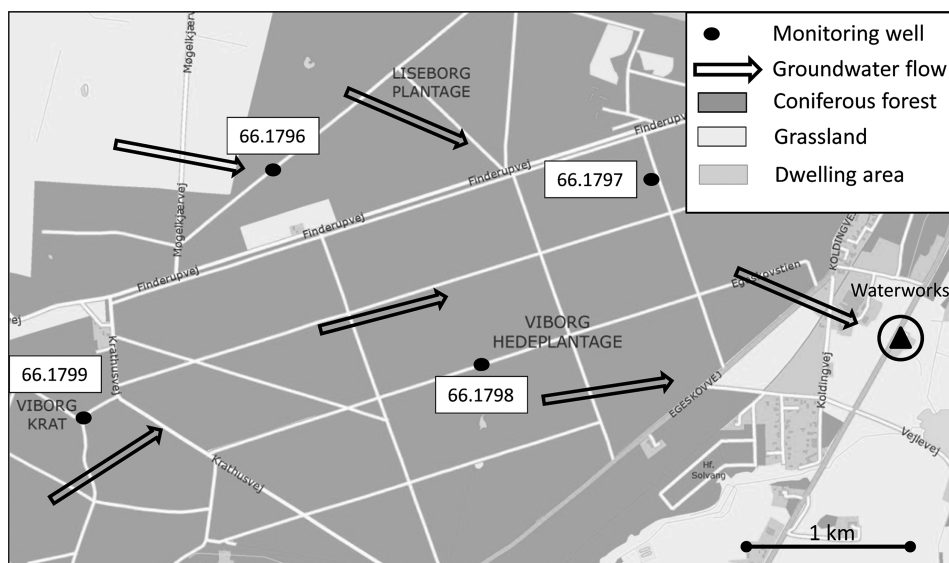


Figure 1. Map of Viborg study site with groundwater flow arrows illustrating the location of the coniferous plantation, the multilevel groundwater wells, the soil gas sampler, and the municipal waterworks.

chloroform is entirely anthropogenic, and that its presence may indicate presence of more toxic halocarbons as well. One major water work already had to seek for a dispensation from the strict rules and others may follow as water works tend to place new water wells in forested areas rather than in farmland in order to avoid problems with nitrate and pesticides. Here, we characterize the carbon isotope composition of natural and anthropogenic chloroform and compare it to chloroform sampled in different aquifers. We show that natural chloroform present in groundwater can be distinguished from anthropogenic chloroform based on its carbon isotope signature. Hence, carbon isotope analysis of chloroform can provide unique insight into the origin of chloroform in the environment and help to take appropriate measures when detecting chloroform in groundwater. While natural chloroform production in forest soils is well established, it is difficult to demonstrate a natural origin of chloroform in groundwater as chloroform can be transported over extended distances from anthropogenic sources. Compounds-specific isotope analysis is increasingly used to distinguish between different anthropogenic sources of chlorinated hydrocarbons in the subsurface because their isotopic signature frequently varies depending on the manufacturer.^{16–19} A similar approach can potentially also be used to distinguish between natural and anthropogenic chloroform because they originate from a different carbon source and are produced by a different mechanism. Industrial chloroform is usually produced from methane which has a typical $\delta^{13}\text{C}$ between -40 and -60% .^{17,20,21} In contrast, naturally produced chloroform originates from natural organic carbon which has $\delta^{13}\text{C}$ around -22 to -30% . However, depending on the pathway, the isotope composition of chloroform may differ from this value due to isotope fractionation during production.^{22,23} Furthermore, the isotopic signature of chloroform may be influenced by degradation of chloroform, for example in anoxic microniches of soil and unsaturated zone or in the saturated zone below the water table. To evaluate if carbon isotope analysis can be used to distinguish between natural and anthropogenic chloroform in groundwater, the $\delta^{13}\text{C}$ values of anthropogenic and natural

chloroform was characterized and compared to chloroform detected in groundwater at four sites.

■ MATERIALS AND METHODS

Field Sites. Viborg Site. Viborg is located in Jutland, Denmark. The study area is situated in a coniferous plantation that has been in place since mid 1800. The study area is underlain by sandy glacial outwash deposits acting as an aquifer with a water table around 5 m below land surface. The soil type is an early podsol profile. Groundwater flows toward the waterworks of Viborg located 2 km down gradient of the study site. At the Viborg waterworks, chloroform was detected since 1995 at a concentration around $1.5 \mu\text{g/L}$. In this study, groundwater samples were taken in four multilevel groundwater monitoring wells where chloroform concentration above $0.1 \mu\text{g/L}$ has been detected in previous studies. The groundwater wells consist of 63 mm diameter HDPE tubes with 1–2 m long screens. The soil gas samplers are 4 cm long brass screens situated in 5–7 depths from 0.5 m bgs to just below the water table. The soil gas screens are situated few meters from the well no. 66.1797, Figure 1.

Tisvilde Site. Tisvilde is located at approximately 60 km north of Copenhagen in Zealand, Denmark. The study area is a forest far from industrial activity. This area was forested in the 19th century to prevent soil drift. The coniferous forest consists mainly of Scots Pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*). The topsoil is constituted of an organic horizon mainly composed of partly degraded needles and branches. The aquifer is constituted of sandy glacial outwash deposits with a water level at around 5 m below surface. The monitoring well has a screen in 11–14 m bgs.

Vellev Site. Vellev is located 27 km east of Viborg in an agricultural area with clayey soils and underlain sand gravel aquifer. The investigated well which has a screen between 31.5 and 37.5 m bgs and is situated adjacent to an industrial landfill for refrigerator waste. The groundwater contains beside chloroform ($\sim 10 \mu\text{g/L}$) high amounts of tetra chloromethane, trichloroethylene and other solvents.

Thisted City Site. The well is situated in the central part of Thisted city in a minor park and is a part of the public

waterworks network. The well intake is between 20 and 35 m below surface and the water has a CFC-model-age of 5 years. The location is adjacent to several former small industries as a car repair and a dry cleaning shop.

Tved Site. Tved Plantation is situated 5 km from the coast 9 km north of Thisted and consists mainly of coniferous forest of spruce and pine planted 110 years ago. The top soil is aeolian sand which is widespread with shifting sand deposits caused by the drifting sand. The sand is underlain by glacial weathered until and the aquifer top about 16 m bgs coincides with the Prequaternary surface of Cretaceous white chalk. Two abstraction wells are equipped with screens from 12 to 18 and 24 to 40 m bgs, respectively.

Groundwater Sampling and Concentration Analysis.

Groundwater samples were obtained using a submersible MP1 pump (Grundfos, Bjerringbro, Denmark) with variable flow rate. For the brass filters a peristaltic pump (Ole Dich, type 111LMT, Hvidovre, Denmark) operated at a flow rate of from 100 to 300 mL/min. After purging the internal volume of the sampling system at least 3 times, 125 mL vials were filled for concentration analysis and 2 L redcap bottles (Schott, Mainz, Germany) for stable carbon isotope analysis. Since groundwater was oxic and chloroform is known to be stable under oxic conditions, no preservatives were added and the samples were stored at 4 °C until analysis. The soil gas samples were collected in 300 and 500 mL stainless steel gas cylinders with valves at both ends using a membrane pump (model 5002, Thomas, Sheboygan, WI). After purging the internal volume of the sampling system and cylinder at least three times, the outlet of the gas cylinder was closed and the cylinders pressurized to around 3 bar to increase the amount of sampling gas.

Chloroform Analysis and CFC Age-Dating. Air samples were analyzed for chloroform, CFCs and other C₁-organohalogens on a gas chromatograph equipped with an ECD detector (GC-8A, Shimadzu, Kyoto, Japan). The analytical procedure was similar to that described by Busenberg and Plummer²⁸ for chlorofluorocarbons (CFC) in age-dating of young groundwater. Briefly, the halocarbons were trapped at -30 °C on a precolumn, which was then heated to 95 °C. Separation of gas constituents was done on a 1.7 m packed column (Poracil-C, Supelco, Bellefonte, PA) at 70 °C. Precolumn back-flush technique was used to complete the analysis of each sample within 11 min. 50 mL of gas sample was used for analysis. The limit of quantification (LOQ) for chloroform was ~10 pptv.

Carbon Isotope Analysis. Since chloroform may occur at low concentration in groundwater, a sensitive analytical method is required. The most sensitive commercial method usually used in groundwater stable isotope studies of VOCs is purge and trap coupled to gas chromatography isotope-ratio mass spectrometry with an estimated detection limit of 2.3 µg/L for chloroform.²⁹ However, such a detection limit is not sufficient to evaluate if low levels chloroform represent a plume fringe or correspond to a natural background. Therefore, a method using a large scale online purge and trap is validated by evaluating the linearity and reproducibility of the method. Stable carbon isotope analysis was carried out with a Thermo Trace gas-chromatograph (GC) coupled to a Thermo Delta Plus XP isotope-ratio mass spectrometer (IRMS) via a GC II interface (all from Thermo Fisher Scientific, San Jose, CA) set to 940 °C. Chloroform in aqueous and gaseous samples was preconcentrated using a modified Velocity XPT Purge & Trap (P&T) system (Teledyne Tekmar Dohrmann, Mason, OH) equipped

with a VOCARB 3000 trap (Supelco, Bellefonte, PA), a forward focusing cell and a dry trap. The P&T system was coupled to the GC via an injector with a cryotrap (Optic 3, ATAS-GL, Vedhoven, The Netherlands) to cool down the initial portion of the capillary column. The GC was equipped with a 60 m × 0.25 mm × 1.8 µm DB-VRX column (Agilent, Santa Clara, CA). For aqueous samples, the purge gas stream was redirected through a 2 L Schott glass bottle equipped with a frit from a gas-washing bottle. Aqueous samples were purged for 20 min at 150 mL/min with leads to a removal of 20% of the dissolved chloroform considering a Henry coefficient of 0.13 (gas/water). The chloroform was trapped on a VOCARB 3000 trap at 30 °C. After a dry purge period of 30 s at 200 mL/min, the chloroform was desorbed for 2 min at 250 °C, transferred to the cryotrap set at -100 °C in splitless mode, and then released to the GC column by rapidly heating the cryotrap to 180 °C. The GC was set to 40 °C for 3 min followed by a ramp of 10 °C/min until a final temperature of 200 °C that was held for 4 min. The column flow was 1.7 mL/min. For gaseous samples, an analogous procedure was used; expect that a stainless steel gas cylinder was integrated into the purge gas circuit rather than a purge vessel. The sample gas was released from the pressurized cylinder at a rate of 150 mL/min to the VOCARB trap followed by purging two times the volume of the cylinder. The isotope ratios are reported in the delta notation relative to the V-PDB standard defined as follows:

$$\delta^{13}\text{C} = \left(\frac{R}{R_{\text{std}}} - 1 \right) \cdot 1000(\text{‰})$$

To validate the system, reference chloroform with a $\delta^{13}\text{C}$ of $-47.9 \pm 0.3\text{‰}$ was analyzed for a concentration range of 0.3–3 µg/L (see Supporting Information, Figure S1). The method detection limit (MDL) was calculated using the moving mean procedure. The samples were measured five times for each concentration. As the difference in $\delta^{13}\text{C}$ between anthropogenic and natural chloroform was expected to be large, a standard deviation ($n = 5$) of $\pm 0.8\text{‰}$ was considered as acceptable to reach lower detection limits. An interval of $\pm 0.8\text{‰}$ was set around the moving mean $\delta^{13}\text{C}$ value. The moving mean $\delta^{13}\text{C}$ value was calculated by including consecutively the $\delta^{13}\text{C}$ value of the next lower concentration as long as the standard deviation was smaller or equal to the specified level ($\pm 0.8\text{‰}$). The MDL corresponds to the lowest concentration for which the mean $\delta^{13}\text{C}$ is within an interval given by the moving mean $\delta^{13}\text{C}$ value $\pm 0.8\text{‰}$. The total uncertainty was characterized by calculating the standard deviation of all $\delta^{13}\text{C}$ values at or above the MDL. Using this approach, a MDL of 0.6 µg/L and a standard deviation of $\pm 0.6\text{‰}$ ($n = 25$) was obtained.

RESULTS AND DISCUSSION

The isotopic signature of industrial chloroform was characterized by analyzing chloroform from several suppliers and taking into account literature values (Table 1). The $\delta^{13}\text{C}$ of industrial chloroform was comprised between -43.2‰ and -63.6‰ ($n = 1-6$; $1\sigma = 0.08-0.39\text{‰}$) consistent with its production from methane.²⁹ The similarities in $\delta^{13}\text{C}$ values between some chloroform suppliers suggest that the samples may come from the same carbon feedstock and similar production processes. It is also possible that chloroform from different suppliers is produced by the same manufacturer.

The isotopic signature of natural chloroform produced in forest soils was constrained based on soil-air samples from a

Table 1. ^{13}C Isotope Signature of Different Industrially Produced Chloroform

$\delta^{13}\text{C}$ (‰/ V-PDB)	$\pm 1\sigma$	<i>n</i>	supplier	purity (%)	ref
-43.2		1			Holt et al., 1997
-43.3		1			Holt et al., 1997
-47.1	0.20	5	Fluka	99.5	this study
-47.9	0.08	5	Alfa Aesar	99	this study
-48.6	0.30	5	Acros Organics	99.8	this study
-51.5	0.22	3	Fisher Scientific	>99	this study
-51.7	0.39	2			Jendrzewski et al., 2001
-63.4	0.22	6	Sigma-Aldrich	99.8	Hunkeler and Aravena, 2000
-63.6	0.14	4	Sigma-Aldrich	99.8	Hunkeler and Aravena, 2000

field site in Viborg, Denmark. At this site, a natural origin of chloroform in soils is well established based on spatial and temporal concentration patterns.^{24–27} Chloroform concentrations and isotope ratios were measured in a soil-gas multilevel sampler reaching to 4 m depth located adjacent to well n° 66–1797, where the highest chloroform concentrations occurred (Figure 1). Maximum chloroform concentrations were detected during summer and autumn at 0.5 m depth (Figure 2)

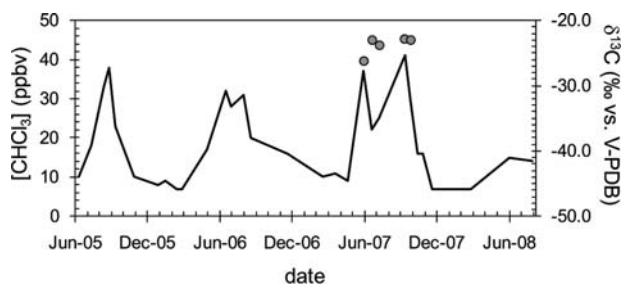


Figure 2. Concentration and carbon isotope signature of the natural chloroform source of Viborg. The black line corresponds to the evolution of the chloroform concentration between June 2005 and August 2008 (modified from Albers et al., 2010). The circles represent the isotope $\delta^{13}\text{C}$ ratios of chloroform.

consistent with a biological production of chloroform in the organic-rich soil zone during the warmer periods of the year.^{25–27} The $\delta^{13}\text{C}$ of chloroform was within a narrow range from -22.8 to -26.2‰ ($n = 2$; $1\sigma = 1.08$ – 1.97‰ , Figure 2), which is close to the typical isotope signature of soil organic carbon (Figure 3) and strongly enriched in ^{13}C compared to industrial chloroform (Figure 2). The somewhat higher standard deviation of these results is a consequence of a change in the P&T-method to use compressed soil air. However, these results confirm that the soil organic matter constitute the main carbon source of natural chloroform.

To demonstrate that carbon isotope analysis can be used to determine the origin of chloroform in groundwater, the $\delta^{13}\text{C}$ of chloroform at five sites was determined using a new method that permits to measure $\delta^{13}\text{C}$ at low concentration levels. Groundwater was collected at the Viborg site where chloroform is known to be produced naturally in the soil organic horizon (see above) and down gradient of an industrial landfill at Vellev, Denmark, where chloroform is likely of anthropogenic origin. In addition, groundwater was also sampled at the

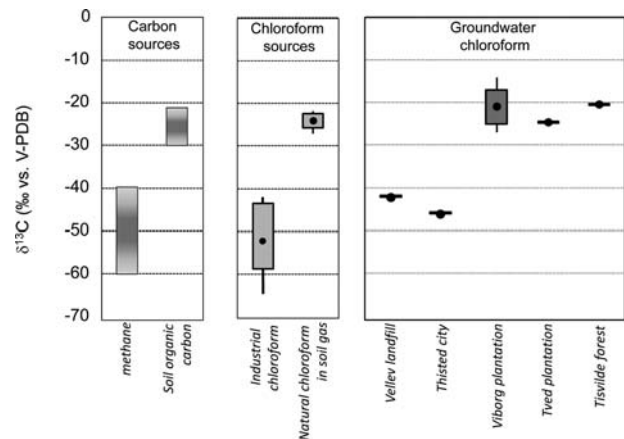


Figure 3. Carbon isotopic signature of carbon and chloroform sources and groundwater chloroform. The gray bars represent the standard deviation around the mean $\delta^{13}\text{C}$ value (black dots) and the vertical black lines correspond to the minimum and the maximum $\delta^{13}\text{C}$ values.

Tisvilde and Tved sites with no identified anthropogenic chloroform sources.

At Viborg, groundwater samples were taken in four multilevel monitoring wells in which chloroform was previously detected (Figure 1). The highest chloroform concentrations were detected in 66–1797 ranging from 0.9 to 4.1 $\mu\text{g/L}$ ($n = 24$) (Figure 4b). The CFC groundwater-age reached 35 years in the

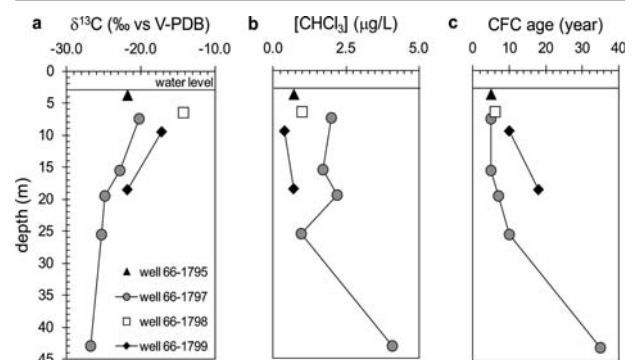


Figure 4. Profile of the $\delta^{13}\text{C}$ values, concentration and CFC age of groundwater at different depths at the Viborg plantation.

deepest sampling point (Figure 4c). The $\delta^{13}\text{C}$ of chloroform at the water table (-22.0‰) corresponded well to the $\delta^{13}\text{C}$ of soil gas chloroform (-22.8 and -26.2‰) demonstrating that chloroform maintains its characteristic isotope signature during transport through the unsaturated zone. The $\delta^{13}\text{C}$ values of chloroform in deeper zones varied between -16.7 and -26.8‰ ($n = 5$) (Figure 4a). The range in $\delta^{13}\text{C}$ chloroform of uppermost groundwater was found within -17 to -29‰ (unpublished data) and the nearly horizontal flowing groundwater from five screens reflect the different infiltration areas of chloroform formation in the forest. At the Tved site, the chloroform concentrations in groundwater between 12 and 18 m bgs. were 1.54–1.86 $\mu\text{g/L}$ ($n = 9$), the $\delta^{13}\text{C}$ between -24.3 and -25.2‰ ($n = 10$), and CFC-groundwater ages approximately 20 years. At the Tisvilde site, the $\delta^{13}\text{C}$ of chloroform was -20.1‰ , (Figure.3).

At the Vellev site, the chloroform concentration in groundwater was 10.7 $\mu\text{g/L}$, which is 2 to 5 times higher

than the concentration measured at the other sites in forested areas. The $\delta^{13}\text{C}$ of chloroform at the Vellev site amounted to -42.1% . At the Thisted site the chloroform concentration was minor than the Vellev site, $<0.2 \mu\text{g/L}$ and the chloroform $\delta^{13}\text{C}$ was -47.0% .

At three of the study sites (Viborg, Tved and Tisvilde), the $\delta^{13}\text{C}$ of groundwater chloroform was close to the values of Viborg soil-gas chloroform indicating a natural origin of chloroform (Figure 3a and 3c). This conclusion is plausible as the three sampling sites were located within spruce and pine plantations, which are associated with soils that are favorable for chloroform production. In contrast, chloroform from the Vellev site had clearly an anthropogenic signature (Figure 3) consistent with its likely origin from an industrial landfill and a concentration higher than at the other three sites. Chloroform was detected in groundwater samples as old as 35 years demonstrating that chloroform can persist over extended periods in oxic groundwater. The persistence of chloroform in groundwater and its high mobility makes it particularly difficult to relate chloroform in groundwater to its source based on concentration data alone as chloroform might be transported over several kilometers. However, the Viborg study indicates that the origin of chloroform can still be determined based on isotope data even if chloroform was transported over an extensive distance. The $\delta^{13}\text{C}$ of chloroform at the Viborg water works down gradient of the plantation with an average CFC-groundwater age of 30 years had a $\delta^{13}\text{C}$ of -24.2% , which is still within the range of the $\delta^{13}\text{C}$ in soil gas at the Viborg plantation.

In summary, the study demonstrates that natural and anthropogenic chloroform have a distinctly different carbon isotope signature that can be related to the carbon source from which chloroform originates, soil organic matter and methane, respectively. The strong difference in $\delta^{13}\text{C}$ makes it possible to clearly identify the origin of chloroform in groundwater even if some changes of the isotope ratios occur during transport. The study demonstrates that chloroform can be naturally present in groundwater at the low microgram level and persist over decades. The isotope method opens new possibilities for a comprehensive assessment of the natural occurrence of chloroform in groundwater. It also helps to take appropriate measures when detecting chloroform in groundwater.

We have in this study demonstrated that it is possible to distinguish between the naturally formed chloroform and industrial produced chloroform using isotopic signature. This has led to the Danish EPA to change the limit of chloroform in groundwater to be used for drinking water from 1 to $10 \mu\text{g/L}$ when the origin of chloroform is a natural source.

■ ASSOCIATED CONTENT

📄 Supporting Information

Additional information as noted in the text. 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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■ REFERENCES

- (1) Squillace, P. J.; Moran, M. J.; Lapham, W. W.; Clawges, R. M.; Zogorski, J. S. Volatile organic compounds in untreated ambient groundwater of the United States, 1985–1995. *Environ. Sci. Technol.* **1999**, *33* (23), 4176–4187.
- (2) Squillace, P. J.; Scott, J. C.; Moran, M. J.; Nolan, B. T.; Kolpin, D. W. VOCs, pesticides, nitrate, and their mixtures in groundwater used for drinking water in the United States. *Environ. Sci. Technol.* **2002**, *36* (9), 1923–1930.
- (3) Juhler, R. K.; Felding, G. Monitoring methyl tertiary butyl ether (MTBE) and other organic micropollutants in groundwater: results from the Danish national monitoring program. *Water, Air, Soil Pollution* **2003**, *149*, 145–161.
- (4) Gribble, G. W. The diversity of naturally produced organohalogenes. *Chemosphere* **2003**, *52*, 289–297.
- (5) Laturmus, F.; Haselmann, K. F.; Borch, T.; Grøn, C. Terrestrial natural sources of trichloromethane (chloroform, CHCl_3) - An overview. *Biogeochemistry* **2002**, *60* (2), 121–139.
- (6) Haselmann, K. F.; Laturmus, F.; Grøn, C. 2002. Formation of chloroform in soil. A year-round study at a Danish spruce forest site. *Water, Air, Soil Poll.* **2002**, *139* (1–4), 35–41.
- (7) Hoekstra, E. J.; Duyzer, J. H.; de Leer, E. W. B.; Brinkman, U. A. T. 2001. Chloroform - concentration gradients in soil air and atmospheric air, and emission fluxes from soil. *Atmos. Environ.* **2001**, *35* (1), 61–70.
- (8) Laturmus, F.; Lauritsen, F. R.; Grøn, C. 2000. Chloroform in a pristine aquifer system: Toward an evidence of biogenic origin. *Water Resour. Res.* **2000**, *36* (10), 2999–3009.
- (9) Hoekstra, E. J.; De Leer, E. W. B.; Brinkman, U. A. T. Natural formation of chloroform and brominated trihalomethanes in soil. *Environ. Sci. Technol.* **1998**, *32* (23), 3724–3729.
- (10) Haselmann, K. F.; Laturmus, F.; Svensmark, B.; Grøn, C. Formation of chloroform in spruce forest soil—Results from laboratory incubation studies. *Chemosphere* **2000**, *41* (11), 1769–1774.
- (11) Walter, B.; Ballschmiter, K. Formation of C1/C2-bromo-chloro-hydrocarbons by haloperoxidase reactions. *Fresenius J. Anal. Chem.* **1992**, *342* (10), 827–833.
- (12) Boyce, S. D.; Hornig, J. F. Reaction pathways of trihalomethane formation from the halogenation of dihydroxyaromatic model compounds for humic acid. *Environ. Sci. Technol.* **1983**, *17*, 202–211.
- (13) Asplund, G.; Christiansen, J. V.; Grimvall, A. A chloroperoxidase-like catalyst in soil—Detection and characterization of some properties. *Soil Biol. Biochem.* **1993**, *25* (1), 41–46.
- (14) Laturmus, F.; Mehrtens, G.; Grøn, C. Haloperoxidase-like activity in spruce forest soil - a source of volatile halogenated organic compounds? *Chemosphere* **1995**, *31* (7), 3709–3719.
- (15) Huber, S. G.; Kotte, K.; Scholer, H. F.; Williams, J. Natural abiotic formation of trihalomethanes in soil: results from laboratory studies and field samples. *Environ. Sci. Technol.* **2009**, *43* (13), 4934–4939.
- (16) Hunkeler, D.; Chollet, N.; Pittet, X.; Aravena, R.; Cherry, J. A.; Parker, B. L. Effect of source variability and transport processes on carbon isotope ratios of TCE and PCE in two sandy aquifers. *J. Contam. Hydrol.* **2004**, *74*, 265–282.
- (17) Jendrzewski, N.; Eggenkamp, H. G. M.; Coleman, M. L. Characterisation of chlorinated hydrocarbons from chlorine and carbon isotopic compositions: scope of application to environmental problems. *Appl. Geochem.* **2001**, *16*, 1021–1031.
- (18) Shouakar-Stash, O.; Frape, S. K.; Drimmie, R. J. Stable hydrogen, carbon and chlorine isotope measurements of selected chlorinated solvents. *J. Contam. Hydrol.* **2003**, *60* (3–4), 211–228.

- (19) Van Wanderdam, E. M.; Frape, S. K.; Aravena, R.; Drimmie, R. J.; Flatt, H.; Cherry, J. A. Stable chlorine and carbon isotope measurements of selected organic solvents. *Appl. Geochem.* **1995**, *10*, 547–552.
- (20) Holt, B. D.; Sturchio, N. C.; Abrajano, T. A.; Heraty, L. J. Conversion of chlorinated volatile organic compounds to carbon dioxide and methyl chloride for isotopic analysis of carbon and chlorine. *Anal. Chem.* **1997**, *69*, 2727–2733.
- (21) Hunkeler, D.; Aravena, R. Determination of stable carbon isotope ratios of chlorinated methanes, ethanes and ethenes in aqueous samples. *Environ. Sci. Technol.* **2000**, *34*, 2839–2844.
- (22) Arnold, W. A.; Bolotin, J.; Von Gunten, U.; Hofstetter, T. B. Evaluation of functional groups responsible for chloroform formation during water chlorination using compound specific isotope analysis. *Environ. Sci. Technol.* **2008**, *42* (21), 7778–7785.
- (23) Bergamaschi, B. A.; Fram, M. S.; Kendall, C.; Silva, S. R.; Aiken, G. R.; Fujii, R. Carbon isotope constraints on the contribution of plant material to the natural precursors of trihalomethanes. *Org. Geochem.* **1999**, *30*, 835–842.
- (24) Albers, C. N.; Hansen, P. E.; Jacobsen, O. S. Trichloromethyl compounds – natural background concentrations and fates within and below coniferous forests. *Sci. Total Environ.* **2010**, *408* (24), 6223–6234.
- (25) Albers, C. N.; Laier, T.; Jacobsen, O. S. Formation, fate and leaching of chloroform in coniferous forest soils. *Appl. Geochem.* **2010**, *25*, 1525–1535.
- (26) Albers, C. N.; Jacobsen, O. S.; Flores, É.M.M.; Pereira, J. S. F.; Laier, T. Spatial variation in natural formation of chloroform in the soils of four coniferous forests. *Biogeochemistry* **2010**, 10533–10550.
- (27) Albers, C. N.; Jacobsen, O. S.; Hansen, P. E. Methodological problems in determining TCAA in soils – the discovery of novel natural trichloroacetyl containing compounds and their interference with a common method for determining TCAA in soil and vegetation. *J. Environ. Monit.* **2010**, *12*, 672–680.
- (28) Busenberg, E.; Plummer, L. N. Use of chlorofluorocarbons (CCl_3F and CCl_2F_2) as hydrologic tracers and age-dating tools: The alluvium and terrace system of central Oklahoma. *Water Resour. Res.* **1992**, *28*, 2257–2283.
- (29) Zwank, L.; Berg, M.; Schmidt, T. C.; Haderlein, S. B. Compound-specific carbon isotope analysis of volatile organic compounds in the low-microgram per liter range. *Anal. Chem.* **2003**, *20*, 5575–5583.