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Position-specific carbon isotope analysis of trichloroacetic acid by gas chromatography/isotope ratio mass spectrometry

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Trichloroacetic acid (TCAA) is an important environmental contaminant present in soils, water and plants. A method for determining the carbon isotope signature of the trichloromethyl position in TCAA using gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) was developed and tested with TCAA from different origins. Position-specific isotope analysis (PSIA) can provide direct information on the kinetic isotope effect for isotope substitution at a specific position in the molecule and/or help to distinguish different sources of a compound. The method is based on the degradation of TCAA into chloroform (CF) and CO2 by thermal decarboxylation. Since thermal decarboxylation is associated with strong carbon isotope fractionation (« = 34.6 0.2%) the reaction conditions were optimized to ensure full conversion. The combined isotope ratio of CF and CO2 at the end of the reaction corresponded well to the isotope ratio of TCAA, confirming the reliability of the method. A method quantification limit (MQL) for TCAA of 18.6 mg/L was determined. Samples of TCAA produced by enzymatic and non-enzymatic chlorination of natural organic matter (NOM) and some industrially produced TCAA were used as exemplary sources. Significant different PSIA isotope ratios were observed between industrial TCAA and TCAA samples produced by chlorination of NOM. This highlights the potential of the method to study the origin and the fate of TCAA in the environment.

Trichloroacetic acid (TCAA) is an important environmental contaminant widely distributed around the world, especially in forest soils.[1–3] TCAA has also been found in remote locations such as in 100-year-old glaciers and firn ice in Antarctica.[4] TCAA can originate from anthropogenic and natural sources. It is known to be a disinfection by-product formed during drinking water chlorination and it has also been detected in effluents from paper manufacturing.[5–7] The atmospheric oxidation of perchlorethylene and 1,1,1-trichloroethane emitted from industries may also lead to the formation of TCAA.[5] TCAA has been widely used many years as a herbicide in agriculture and as a polymerization catalyst in the chemical industry.[5] This compound is of environmental concern because of its high phytotoxicity, causing chlorosis. In addition, TCAA may have direct effects on human health such as toxic and mutagenic effects on the liver and heart.[8] With regard to natural sources of TCAA, studies by Matucha *et al*.[9] and Heal *et al*.[10] suggest that TCAA may be formed in soil via chlorination of organic material, analogous to the production of chloroacetic acids from humic substances during drinking water treatment.[11] Laboratory studies suggest that TCAA is produced when humic or fulvic acids are incubated with chloroperoxidase (CPO) in the presence of chloride and H_2O_2.[12,13]

Compound-specific isotope analysis (CSIA) is increasingly used to evaluate the source and fate of different classes of organic compounds such as chlorinated solvents (e.g. PCE, TCE and DCE), PCBs and aromatic compounds (e.g. BTEX and PAHs).[14] As the isotopic composition of a compound often varies as a function of its source, CSIA can be used to distinguish different sources of a given contaminant. It can be also used to study the fate of organic compounds in the environment (e.g. biodegradation, vaporization, diffusion).[14]

CSIA has been little used for evaluating the origin and fate of TCAA, partly due to the lack of a simple analytical method. Because of the low Henry's constant ($7.4 \cdot 10^4$ mol/kg atm[15]) and high water solubility (81.7 g/L H_2O at 20 °C, Sigma-Aldrich) of TCAA, aqueous samples of TCAA cannot be analyzed by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) using common extraction methods for volatile organic compounds.[15] Wong *et al*.[16] have developed an analytical procedure to measure the $\delta^{13}C$ values of chloroacetic acids using derivatization with acidic methanol with a known isotopic signature. In this method chloroacetic acids are transformed into chloroacetic acid methyl esters, extracted with pentane and then concentrated by gentle blowdown using nitrogen gas before analysis. Although the effect of derivatization on the isotope ratio can be accounted for using a carbon mole balance, this method is labor-intensive. An alternative method of sample pretreatment is thermal decarboxylation that leads to the production of chloroform (CF) which can be easily analyzed by GC/C/IRMS. Albers *et al*.[17] have demonstrated that the thermal decarboxylation of TCAA has to be carried out at pH <3 to minimize the interference from trichloroacetyl-containing compounds. At pH above 3 trichloroacetyl-containing compounds can be hydrolyzed and can also form CF and carboxylic acids. The thermal decarboxylation method provides position-specific isotope ratios. This can be an advantage when fingerprinting different TCAA sources

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as they may have a same average isotope ratio value ($\delta^{13}C_{CSIA}$) but different position-specific isotope ratios ($\delta^{13}C_{PSIA}$). Furthermore, it is possible to access directly the effect of isotope substitution at the trichloromethyl position on the reaction rate (i.e. hydrolysis).

Here, an analytical procedure to determine the carbon position-specific isotope signature of the trichloromethyl group in TCAA using thermal decarboxylation is described. Moreover, this study examines the isotopic composition of TCAA produced by chlorination of natural organic matter (NOM) to evaluate the ability of the method to study TCAA produced by different processes.

EXPERIMENTAL

Chemicals and reagents

The following chemicals were used as received: trichloroacetic acid (Sigma-Aldrich, St Louis, MO, USA, 99.0% purity; Acros Organics, Geel, Belgium, 99%; Fisher Scientific, Wohlen, Switzerland, analytical reagent grade), sodium hypochlorite solution (Sigma-Aldrich, available chlorine ≥4%), phosphoric acid (Fluka, Seelze, Germany, 85%), sodium dihydrogen phosphate monohydrate (Merck, >99%), sodium sulfite (Sigma-Aldrich, >98%), nitric acid (Carlo Erba, Milan, Italy, 65%), Chloroperoxidase from *Caldariomyces fumago* (Sigma-Aldrich, >10000 U/mL), potassium chloride (Fluka, puriss. p.a.), hydrogen peroxide (Fluka, pursi. p.a.). Ultrapure water (18.2MΩ cm at 25 °C, Direct-Q UV-3; Millipore, Billerica, MA, USA) was used to prepare the aqueous solutions. The following humic substances from the International Humic Substance Society (St. Paul, MN, USA) and soil were used for TCAA formation experiments: Suwannee river NOM (IHSS, 1R101N), Nordic reservoir NOM (IHSS, 1R108N), Pahokee peat humic acid (IHSS, 1R103H), Elliot soil humic acid (IHSS, 1S102H) and coniferous forest soil (F-horizon, Tisvilde, Denmark).

Quantification of reaction yield and isotope fractionation during thermal decarboxylation

Vials of 42 mL volume were completely filled with aqueous solutions of industrial TCAA (Sigma Aldrich) prepared from a stock solution with water acidified at pH 2. The initial concentrations of TCAA were varied (from ~10 mg/L to ~50 μg/L) taking into account the reaction kinetics and decarboxylation time such that 17 nmol CF was expected to be formed in 42 mL of sample.[18] As thermal decarboxylation of TCAA is a slow process at room temperature, the experiment was conducted at 65 °C using a thermostatted water bath. The decarboxylation was conducted at a low temperature to avoid an overpressure in the vials. The thermal decarboxylation was stopped after 2, 4, 8, 24, 32, 48, 56, 72, 96 and 120 h by cooling the vials to 4 °C. Based on the kinetic constants determined by Zhang and Minear,[19] the conversion of TCAA into CF is expected to be nearly complete after 120 h of reaction. The $\delta^{13}C_{PSIA}$ value of the CF released by thermal decarboxylation of TCAA was measured in triplicate each time using the purge-and-trap method described below.

To establish an isotope balance of the decarboxylation products for quality control, the $\delta^{13}C$ isotope ratio of the CO_2 produced during the decarboxylation of TCAA was measured using a glass gas trap (Fig. 1); 10 mg of pure TCAA (Sigma Aldrich) were introduced into the gas trap. The atmospheric CO_2 present in the gas trap was purged for 2 min using a ME2 vacuum pump (Vacuubran, Wertheim, Germany) and the air was replaced by pure argon. This step was repeated three times in order to ensure that no traces of atmospheric CO_2 remained in the gas trap. The gas trap was

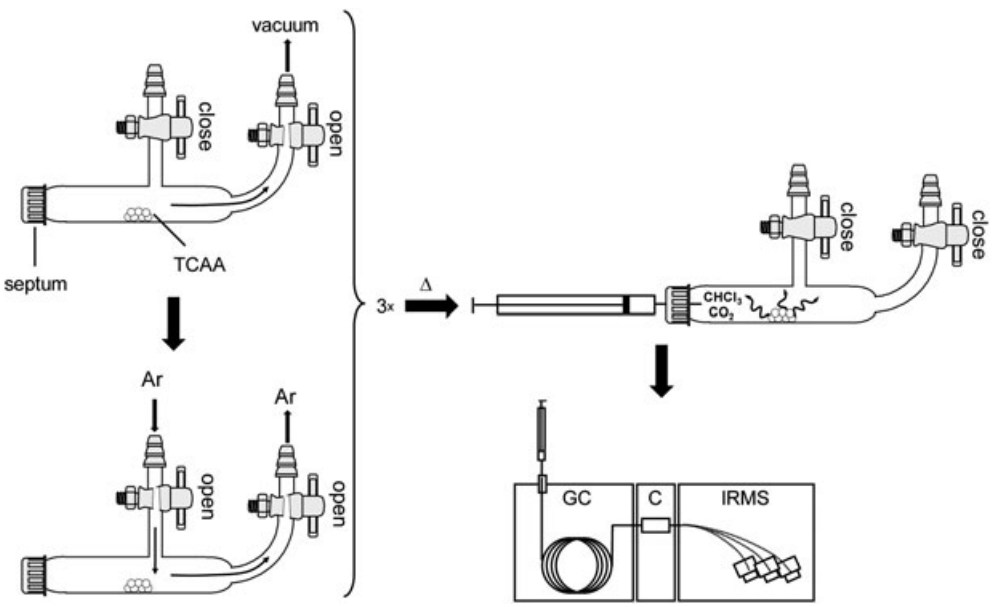

Figure 1. Procedure for the carbon isotope analysis of CO_2 released by thermal decarboxylation of TCAA.

placed in an oven at 90 °C for 24 h and the CO_2 produced was sampled through a septum with a 2 mL gas-tight syringe and analyzed by GC/C/IRMS.

Determination of enrichment factor

Isotope fractionation associated with the release of CF can be quantified by the following isotope fractionation factor α:

$$\alpha = \frac{\frac{d^{13}CF}{d^{12}CF}}{\frac{^{13}TCM}{^{12}TCM}} \quad (1)$$

where $d^{13}CF$ and $d^{12}CF$ are the amounts of instantaneously produced CF with ^{13}C and ^{12}C, respectively; and ^{13}TCM and ^{12}TCM are the remaining amounts of trichloromethyl-carbon with ^{13}C and ^{12}C, respectively. Isotope fractionation can also be expressed in terms of an enrichment factor ε, which is defined by:[14]

$$\varepsilon = (\alpha - 1)\cdot 1000 \quad (2)$$

The $\delta^{13}C$ isotope ratio of the accumulating CF that is measured in the experiment, $\bar{R}_{CF}$, is expected to evolve according to the following relationship:

$$\bar{R}_{CF} = R_{TCM,f=0}\cdot\frac{1 - f^{\left(\frac{\varepsilon}{1000}+1\right)}}{1-f} \quad (3)$$

where $R_{TCM,f=0}$ is the final isotope ratio of the trichloromethyl-carbon position and f the remaining fraction of TCAA ($f = C/C_0$). The CF concentration was determined on the basis of the peak area of m/z 44 using a five-points calibration with external standards and f was then derived from the CF concentrations. The CF peak area of m/z 44 is proportional to the TCAA concentration ($r^2 = 0.9985$). The measured $\delta^{13}C$ isotope ratios of the accumulating CF were fitted with Eqn. (3) by varying the enrichment factor ε such that the sum of squared residuals was minimized (least-squares method). Since only one carbon atom is present in CF, the fractionation factor α can be directly related to the kinetic isotope effect (KIE) according to:

$$KIE = \frac{1}{\alpha} \quad (4)$$

Production of TCAA from NOM

TCAA was formed by chlorination of forest soil and four humic substances with sodium hypochlorite: 40 mg of soil or 5 mg of humic substances was added to a 42 mL vial containing 40 mL of 100 mM phosphate buffer, the reaction was initiated by adding 100 µL of an aqueous solution of 0.02 M NaOCl and the remaining volume was completed with pure water. The chlorination was carried out at pH 4 in order to simulate the conditions encountered in forest soils. The vials were sealed with Teflon septum caps and agitated for 24 h at room temperature (~25 °C). After 24 h, the samples were quenched with 100 µL of sodium sulfite solutions (100 g/L) to stop the reaction.

Forest soil and Elliott soil humic acids were also chlorinated with CPO and potassium chloride: 40 mg of soil or 5 mg of humic acid was added to 42 mL vials filled with 40 mL of 100 mM pH 4 phosphate buffer solution containing 0.375 mg/L of KCl. Then 5 µL of an aqueous suspension of CPO of $>10^4$ units/mL was added and the vials were stirred for 30 min. Subsequently, 1 mL of 0.0129 M H_2O_2 was added gradually over a 1.5 h interval. The solution was stirred for 1 h, and 1 mL of H_2O_2 solution was then added over a second 1.5 h interval.

Pretreatments of TCAA samples

The pH of the samples containing TCAA produced by chlorination of NOM was adjusted to ≥12 and the samples were purged for 30 min with pure N_2 to remove CF formed by the hydrolysis of trichloroacetyl-containing compounds.[17] The pH of the samples containing industrial TCAA or TCAA formed by chlorination of NOM was then set to ≤2 with concentrated HNO_3 and the vials were filled with pure water such that no headspace remained. Finally, the vials were heated in an oven at 65 °C for 120 h to completely convert the TCAA into CF by thermal decarboxylation. All samples were stored in the dark at 4 °C until analysis by purge-and-trap GC/C/IRMS. The $\delta^{13}C_{PSIA}$ values of the trichloromethyl position of TCAA produced by chlorination of organic matter were compared with the $\delta^{13}C_{PSIA}$ values of synthetic TCAA from different suppliers.

^{13}C analysis of CF from thermal decarboxylation of TCAA

The $\delta^{13}C$ isotope ratios of CF produced during the TCAA thermal decarboxylation were measured with a Thermo Trace gas chromatograph coupled to a Thermo Delta XP isotope ratio mass spectrometer via a GC Combustion III interface (Thermo Fisher Scientific, San Jose, CA, USA). The gas chromatograph was equipped with a cryogenic focuser (Optic 3, ATAS-GL, Veldhoven, The Netherlands) and coupled to a Velocity XPT purge-and-trap concentrator (Teledyne Tekmar Dohrmann, Mason, OH, USA) with an AquaTek70 liquid autosampler (Teledyne Tekmar Dohrmann). Aqueous samples (25 mL) were purged with a N_2 flow of 40 mL/min and volatiles were trapped on a VOCARB 3000 trap (Supelco, Bellefonte, PA, USA) at 35 °C. After the extraction step, the trap was heated to 250 °C for 3 min. Chloroform was thermally desorbed and transferred to the gas chromatograph. The chromatographic separation was carried out with a DB-VRX column (60 m, 0.25 mm, 1.4 µm; Agilent, Santa Clara, CA, USA). The column flow of the gas chromatograph was maintained constant at 1.7 mL/min. The following GC oven temperature program was used: 6 min at 40 °C, 10 °C/min to 175 °C, 175 °C held for 1 min. The cryogenic focuser was set to −100 °C for 3 min with liquid N_2. The oxidation and reduction reactors of the combustion interface were maintained, respectively, at 940 °C and 650 °C. In order to maximize the accuracy of the measured $\delta^{13}C$ values, the samples were diluted to obtain constant peak amplitudes. The purge efficiency was determined by measuring the peak area of m/z 44 for different purge times and was compared with the theoretical extraction yield predicted by the following equation:[20]

$$\frac{Area^{total}_{mass\,44}}{Area^{t}_{mass\,44}} = 1 - e^{\left(-\frac{K_{aw}G}{V_w}\right)t} \cdot 100(\%) \quad (5)$$

where $Area^{total}_{mass44}$ and $Area^{t}_{mass44}$ are, respectively, the peak area of *m/z* 44 for total extraction and at a specified time *t*. V_w is the volume of the aqueous sample in mL, *G* the purge gas flow rate in mL/min and K_{aw} the air-water partition constant ($K_{aw}(CHCl_3) = 0.13$ at 20 °C).[20] For the determination of $\delta^{13}C$ values of CO_2 the gas chromatograph was fitted with an injection valve with a 250 µL loop (VICI, Houston, TX, USA) and a Rt-QSplot column (30 m, 0.32 mm i.d., 10 µm; Restek, Bellefonte, PA, USA). The GC oven temperature was maintained at 35 °C.

The $\delta^{13}C$ values of pure solid TCAA samples were determined in triplicate using an elemental analyzer coupled with a MAT Delta S stable isotope mass spectrometer (Finnigan, Bremen, Germany). All the $\delta^{13}C$ values were reported in ‰ relative to Vienna PeeDee Belemnite (V-PDB) defined as:

$$\delta^{13}C = \left(\frac{R}{R_{std}} - 1\right) \cdot 1000\left(^{0}/_{00}\right) \quad (6)$$

RESULTS AND DISCUSSION

Evaluation of decarboxylation time and enrichment factor

The decarboxylation yields of TCAA (Sigma-Aldrich) at different reaction times are shown in Fig. 2(a). TCAA is completely converted into CF after 120 h of reaction. At the beginning of the decarboxylation reaction, the CF formed is strongly depleted in ^{13}C (Fig. 2(b)). Afterwards the CF becomes progressively enriched in ^{13}C as the reaction proceeds. After 120 h, the isotope signature of CF ($\delta^{13}C_{PSIA} = -40.4 \pm 0.5$‰, n = 3) is similar to the $\delta^{13}C_{CSIA}$ values of the TCAA used for the experiment ($\delta^{13}C_{CSIA} = -41.3 \pm 0.2$‰, n = 3).The carbon isotope ratio of the CO_2 generated from complete thermal decarboxylation of TCAA is -41.8 ± 0.9‰ (n = 6). The $\delta^{13}C$ value of TCAA calculated based on the $\delta^{13}C_{PSIA}$ values of the trichloromethyl and carboxyl positions (-41.1 ± 1.0‰) corresponds well to the measured $\delta^{13}C$ value using the EA/IRMS method (-41.3 ± 0.2‰), thus confirming the accuracy of the analytical methods.

The isotope enrichment factor obtained using Eqn. (3) (Fig. 3) is $\varepsilon = -34.6 \pm 0.2$‰ ($r^2 = 0.9878$) corresponding to a KIE = 1.036 ± 0.002. Hence, thermal decarboxylation is associated with a normal kinetic isotope effects (KIE >1). The calculated KIE value is consistent with the Streitwieser semi-classical limit for the isotope effect for cleavage of a C–C bond ($KIE_{C\text{-}C} = 1.049$).[14] The somewhat smaller KIE values calculated for the thermal decarboxylation may be an indication that in the transition state the C–C bond is not completely broken. Furthermore, it is expected that C–Cl bonds are strengthened in the transition state, leading to an inverse secondary KIE that partly offsets the primary KIE. The measured KIE is in the same range as the KIE observed by Lindsay *et al*.[21] for thermal decarboxylation of malonic acid, and is within the typical range KIE = 1.03–1.06 of

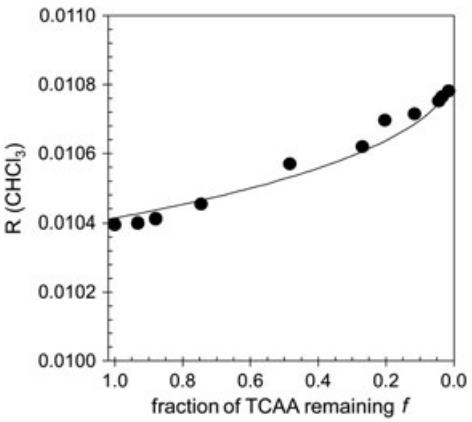

Figure 3. Measured (black dots) and modeled (black line) carbon isotope signatures of accumulated CF.

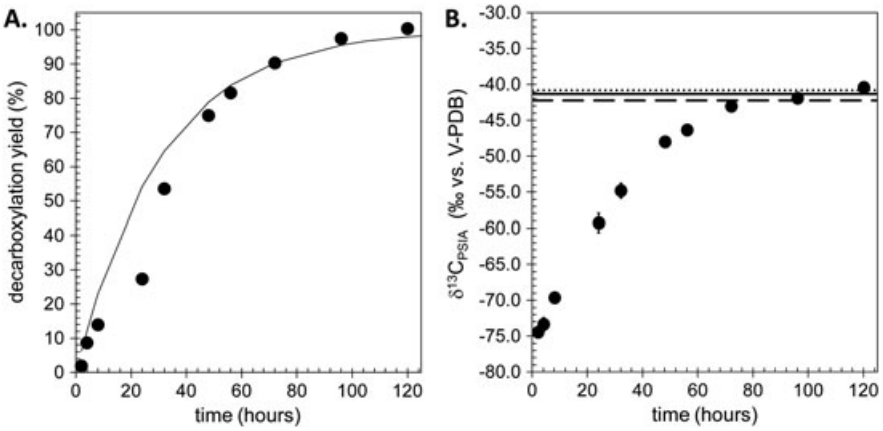

Figure 2. (a) Measured (black dots) and modeled (black line) decarboxylation yields calculated using the decomposition rate constants determined by Zhang and Minear.[19] (b) Measured (black dots) isotope signature of chloroform released during the thermal decarboxylation of TCAA versus time. The full line corresponds to the $\delta^{13}C$ value of TCAA, the dashed line to the $\delta^{13}C$ value of the carboxyl position in TCAA, and the dotted line to the calculated $\delta^{13}C$ value of the trichloromethyl position in TCAA. The error bars correspond to the standard deviation.

reactions for which the decarboxylation is entirely rate-determining.[22–24] Therefore, the KIE of the thermal decarboxylation of TCAA is consistent with the chemical mechanism proposed by Atkins *et al*.[25] where, at pH ~2, TCAA ($pK_a = 0.64$) is deprotonated and the C–C bond of the trichloroacetate anion is cleaved in a rate-limiting step. The heterolytic cleavage of the C–C bond leads to the formation of CO_2 and the trichloromethanide ion which is rapidly converted into CF by protonation.

Determination of purge time and method detection limit

The purge-and-trap extraction efficiency for CF is quite well predicted by the dynamic phase equilibrium model ($r^2 = 0.964$, Fig. 4(a)). The peak area of *m/z* 44 reaches a maximum at between 10 and 15 min and decreases for purge times longer than 15 min. This decrease suggests chloroform breakthrough in the trap. The shift to a lower $\delta^{13}C_{PSIA}$ value at 20 min purge time could originate from this breakthrough as heavy isotopologues might elute preferentially from the trap (Fig. 4(b)).[20] For purge times between 5 and 15 min, constant $\delta^{13}C_{PSIA}$ values are observed. On the basis of these measurements, 10 min is an optimal purge time as high accuracy and high sensitivity are obtained.

The method quantification limit (MQL) was determined using the method developed by Jochmann *et al*.[20] using standards with different concentrations of TCAA prepared from a stock solution. The reproducibility was determined by calculating the mean and standard deviation of all $\delta^{13}C$ values for which the concentration is equal to and above the calculated MQL. A MQL of 18.6 μg/L (5 nmol carbon) and a reproducibility expressed as standard deviation (Fig. 5) of ±0.5‰ (n = 12) are obtained for CF released from TCAA. The calculated MQL value is very close to the MQL determined by Jochmann *et al*.[20] for chlorinated and brominated methanes using a purge-and-trap method. According to McCulloch,[7] the concentrations of TCAA in soils are very variable and, while 60% of the determinations were less than 0.5 μg/kg, the remainder spanned a wide range (up to 150 μg/kg). The concentrations of TCAA in water are also very variable and range between 0.009 μg/L (e.g. natural water) and 7600 μg/L

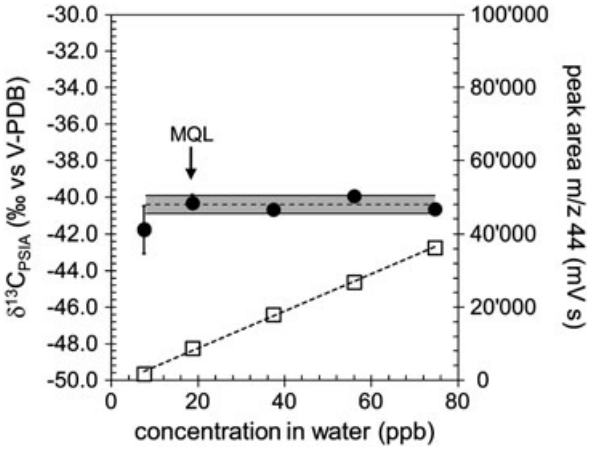

Figure 5. $\delta^{13}C_{PSIA}$ values of TCAA (black dots), peak area of *m/z* 44 (white squares), mean $\delta^{13}C_{PSIA}$ value of TCAA (dashed line) and interval of ±0.5‰ around it (full lines).

(e.g. industrial effluent).[7] Therefore, position-specific isotope analysis should be possible on a wide range of samples. However, a lower quantification limit could be achieved by using a larger sample volume or an anion exchanger to preconcentrate the aqueous TCAA.

Demonstration of applicability

TCAA was produced by the chlorination of NOM with hypochlorite and by CPO-induced chlorination. The trichloromethyl position of TCAA produced from soil and humic substances with hypochlorite gives $\delta^{13}C_{PSIA}$ values of between −23.9 and −32.3‰ (Table 1). These isotopic ratios (mean $\delta^{13}C_{PSIA} = -27.8 \pm 0.5$‰) are in the same range as the isotope signature of soil and humic substances (mean $\delta^{13}C_{precursor} = -26.5 \pm 0.1$‰) used in the experiments and are not influenced by the quantity of the hypochlorite added for the chlorination (Fig. 6).[26] Except for the TCAA produced from Nordic reservoir NOM, all the $\delta^{13}C_{PSIA}$ values of the trichloromethyl position of TCAA are slightly depleted in ^{13}C compared with the precursor isotopic composition. This

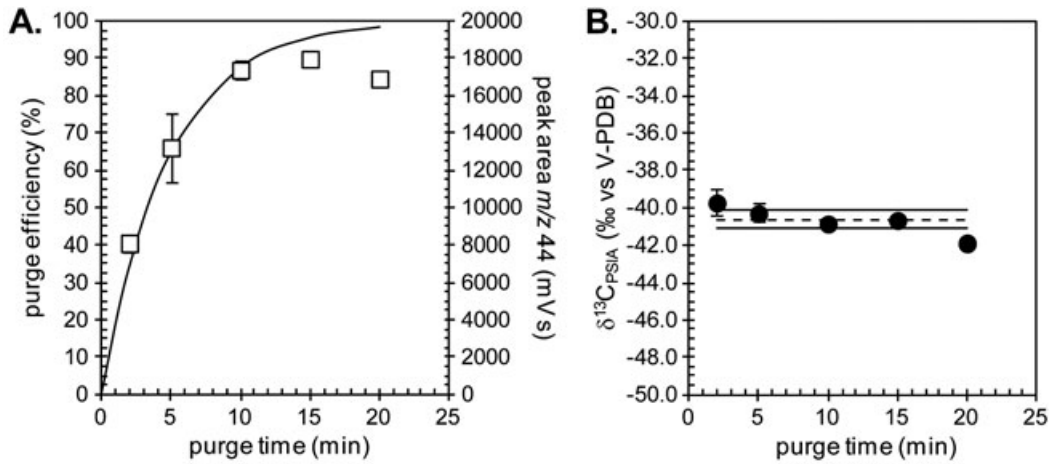

Figure 4. (a) Measured (white squares) and theoretical (solid line) purge efficiency during CF extraction. (b) $\delta^{13}C_{PSIA}$ values of the extracted CF (black dots), mean $\delta^{13}C_{PSIA}$ value of the trichloromethyl position (dashed line) and interval of ±0.5‰ around it (full lines).

Table 1. $\delta^{13}C_{CSIA}$ values (‰) of different TCAA sources and precursors. $\delta^{13}C_{PSIA}$ values (‰) of the trichloromethyl position of different industrial TCAA and TCAA produced by chlorination of NOM

	CSIA (EA/IRMS)				PSIA (GC/C/IRMS)	
	$\delta^{13}C_{precursor}$	SD	$\delta^{13}C_{TCAA}$	SD	$\delta^{13}C_{trichloromethyl}$	SD
Sample	(‰ V-PDB)	(‰ V-PDB)	(‰ V-PDB)	(‰ V-PDB)	(‰ V-PDB)	(‰ V-PDB)
Industrial TCAA						
Fisher Scientific	–	–	−37.9	±0.2	−38.9	±0.7
Acros Organics	–	–	−39.5	±0.3	−43.8	±0.1
Sigma-Aldrich	–	–	−41.3	±0.2	−40.4	±0.5
Chlorination with NaOCl						
Pahokee peat	−26.5	±0.1	–	–	−27.0	±0.4
Nordic reservoir	−27.8	±0.1	–	–	−27.4	±0.5
Suwannee river	−28.0	±0.1	–	–	−32.3	±0.5
Elliott soil	−22.6	±0.1	–	–	−23.9	±0.4
Forest soil	−27.5	±0.1	–	–	−28.5	±0.4
Chlorination with CPO						
Elliott soil	−22.6	±0.1	–	–	−20.2	±0.8
Forest soil	−27.5	±0.1	–	–	−27.3	±0.3

offset is probably due to a difference between the isotopic composition of functional groups involved in the reaction and the bulk isotopic ratios of the humic substances. These $\delta^{13}C$ values are in the same range as the results obtained by Bergamaschi *et al.*[27] ($\delta^{13}C_{CF} = -30.1‰$, $\delta^{13}C_{NOM} = -26.2‰$), showing that the CF produced upon chlorination of agricultural water drainage is slightly depleted in ^{13}C compared with the isotope signatures of dissolved organic matter. The TCAA produced by CPO-induced chlorination of forest soil and humic acid is slightly enriched in ^{13}C compared with the isotopic composition of the precursors and TCAA produced with hypochlorite. The small difference in the $\delta^{13}C_{PSIA}$ values observed between the enzymatic and the non-enzymatic chlorination may be explained by a different degree of reaction advancement between the CPO-induced ($[CF]_{CPO}$: 10–40 µg/L) and non-enzymatic chlorination ($[CF]_{NaOCl}$: 80–150 µg/L). The relatively good agreement of the $\delta^{13}C$ values for CPO-induced and non-enzymatic chlorination is consistent with the hypothesis that CPO reacts with H_2O_2 and oxidizes chloride to hypochlorite which in turn chlorinates the NOM.

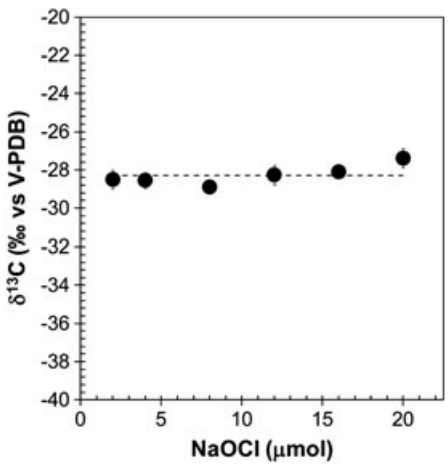

Figure 6. $\delta^{13}C_{PSIA}$ values of the extracted TCAA produced from forest soil with different amount of hypochlorite (black dots), mean $\delta^{13}C_{PSIA}$ value of the trichloromethyl position (dashed line).

The $\delta^{13}C_{PSIA}$ values of the three industrial TCAA samples ranges between −38.9 and −43.8‰, and hence are strongly depleted in ^{13}C compared with the TCAA produced by enzymatic and non-enzymatic chlorination of NOM (Table 1). These relatively low $\delta^{13}C_{PSIA}$ values may result from the use of ^{13}C-depleted reactant during the synthesis of TCAA and the purification processes (e.g. re-crystallization, extraction) inducing isotope fractionation. The variation of the $\delta^{13}C_{PSIA}$ values among the different suppliers suggests that the TCAA samples have been produced from different carbon feed stock with different isotopic signature.[16] While the samples of industrial TCAA analyzed in the present study have distinctly different $\delta^{13}C$ values from that of TCAA from NOM, three TCAA samples analyzed by Wong *et al.*[16] have $\delta^{13}C_{CSIA}$ values (between −27.5 and −29.0‰) that overlap with that of TCAA produced from NOM. Hence, when using isotope analysis for TCAA source identification, it is important to test the plausibility of the results with additional information such as concentration patterns, in the same way as for industrial solvents studies, where different sources can sometimes also have similar carbon isotope signatures. Table 1 shows the $\delta^{13}C_{PSIA}$ and $\delta^{13}C_{CSIA}$ values of three synthetic TCAA samples measured by GC/C/IRMS and EA/IRMS, respectively. While the TCAA samples from Sigma-Aldrich and Fisher Scientific only show small differences between the $\delta^{13}C_{PSIA}$ and $\delta^{13}C_{CSIA}$ values ($\Delta\delta^{13}C_{Sigma\text{-}Aldrich} = 0.9‰$ and $\Delta\delta^{13}C_{Fisher\ Scientific} = 1.0‰$) indicating little intramolecular $\delta^{13}C$ variations, the difference is substantial for the TCAA sample from Acros Organics ($\Delta\delta^{13}C_{Acros\ Organics} = 4.3‰$). The occurrence of intramolecular variations in $\delta^{13}C$ values suggests that PSIA can potentially provide additional discrimination between TCAAs from different sources.

CONCLUSIONS

This study demonstrates that thermal decarboxylation combined with isotope analysis of the released CF can be potentially used as a routine method for carbon isotope analysis of TCAA. This novel method makes it possible to determine with high precision the carbon isotope signature of the trichloromethyl position of TCAA at the ppb level without labor-intensive pretreatment. Hence, the study provides the basis for a more widespread application of isotope analysis to evaluate the origin and fate of TCAA in the environment. The wide range of carbon isotope signatures observed in this study underlines the potential of the method for distinguishing different sources of TCAA although further work is needed to characterize the effect of different processes on the TCAA isotope composition.

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