Chemical synthesis of new chlorine isotope standard materials and characterization via complementary mass spectrometry methods*

Christina Lihl[†], Julian Renpenning[∫], Steffen Kümmel[∫], Faina Gelman[‡], Heide K. V. Schürner[†], Martina Daubmeier[†], Benjamin Heckel[⍰], Aileen Melsbach[†], Anat Bernstein[§], Orfan Shouakar-Stash[ǀ], Matthias Gehre[∫], Martin Elsner*[†⍰]

[†] Institute of Groundwater Ecology, Helmholtz Zentrum München, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

[∫] Department of Isotope Biogeochemistry, Helmholtz Centre for Environmental Research – UFZ, Permoserstraße 15, 04318 Leipzig, Germany

[‡] Geological Survey of Israel, 32 Yeshayahu Leibowitz St., 9692100 Jerusalem, Israel

[⍰] Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Marchionistraße 17, 81377 München, Germany

[§] Zuckerberg Institute for Water Research, Department of Environmental Hydrology and Microbiology, Ben-Gurion University of the Negev, 84990 Sede Boqer, Israel

[ǀ] Department of Earth Sciences, University of Waterloo, 200 University Avenue, Waterloo, Ontario, Canada N2L 3G1

ABSTRACT: Increasing applications of compound-specific chlorine isotope analysis (CSIA) emphasize the need for chlorine reference standards that bracket a wider range of isotope values in order to ensure accurate results. With one exception (USGS38), however, all international chlorine isotope reference materials (chloride and perchlorate salts) fall within the narrow range of one per mille. Furthermore, compound-specific working standards are required for chlorine CSIA, but are not available for most organic substances. We took advantage of isotope effects in chemical dehalogenation reactions to generate (i) silver chloride (CT16) depleted in $^{37}Cl/^{35}Cl$ and (ii) compound-specific standards of the herbicides Acetochlor and S-Metolachlor (Aceto2, Metola2) enriched in $^{37}Cl/^{35}Cl$. Calibration against the international reference standards USGS38 (-87.90 ‰) / ISL-354 (+0.05 ‰) by complementary methods (gas chromatography - isotope ratio mass spectrometry, GC-IRMS versus gas chromatography - multi-collector inductively coupled plasma mass spectrometry, GC-MC-ICPMS) gave a consensus value of $\delta^{37}Cl_{CT16}$ = -26.82 ± 0.18 ‰. Preliminary GC-MC-ICPMS characterization of commercial Aceto1 and Metola1 versus Aceto2 and Metola2 resulted in tentative values of $\delta^{37}Cl_{Aceto1}$ = 0.29 ± 0.29 ‰, $\delta^{37}Cl_{Aceto2}$ = 18.54 ± 0.20 ‰, $\delta^{37}Cl_{Metola1}$ = -4.28 ± 0.17 ‰ and $\delta^{37}Cl_{Metola2}$ = 5.12 ± 0.27 ‰. The possibility to generate chlorine isotope standards with pronounced shifts in isotope values offers a much-needed basis for accurate chlorine CSIA.

* Please note title change upon publication

Isotopes - atomic nuclei that are identical in their chemical properties, but show differences in their atomic mass – may be present in varying proportions. Stable isotope ratios are typically expressed in the δ-notation relative to a common international reference material (see Equation 1). This has the advantage that values, when measured in different laboratories against the same reference material, are comparable on an absolute scale[1,2].

$$\delta^{h}E = [({}^{h}E/{}^{l}E)_{Sample} - ({}^{h}E/{}^{l}E)_{Reference}] / ({}^{h}E/{}^{l}E)_{Reference} \quad (1)$$

$\delta^{h}E$ refers to the isotope value of an element E and $({}^{h}E/{}^{l}E)$ to the absolute ratio of the respective heavy (h) and light (l) isotopes. Positive delta values imply an enrichment and negative delta values indicate a depletion of heavy relative to light isotopes when compared to the international reference standard[3,4]. Isotope ratios are used in a wide field of applications. In archaeology, stable isotope ratios inform about prehistoric lifestyle and diet[5]; in food sciences they serve to test the quality and the origin of foods[6]. In forensic science, isotope analysis can help trace the production site of drugs[7] and in competitive sports it can reveal doping violations[8]. Isotope analysis is equally important in the field of environmental sciences where environmental contaminants threaten the quality of ground water resources. By analyzing isotope ratios of single compounds, compound-specific isotope analysis (CSIA) is able to allocate a contaminant to a certain source[9]. In addition, CSIA can help to detect and quantify isotope fractionation to trace degradation processes of environmental contaminants. Since bonds of molecules with heavy vs. light isotopes are transformed at different rates, isotope ratios change during degradation. Hence, isotope analysis has the potential to identify degradation of contaminants even if no metabolites can be detected. As isotope effects are reaction-specific, isotope ratio analysis of the parent compound may in addition deliver information about chemical transformation pathways - even without metabolite analysis [2,4,10–12].

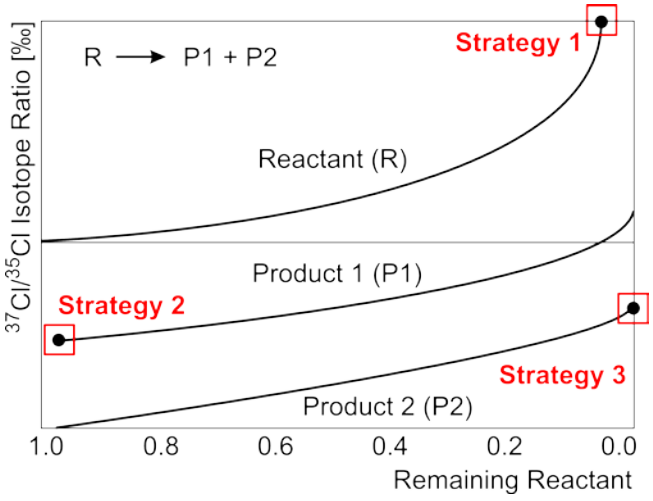
Chlorine isotope analysis ($^{37}Cl/^{35}Cl$) has increased in importance with its role in deciphering central geochemical and biological processes. Since chloride is one of the most abundant anions in geological fluids, its isotopes were measured early on to obtain information about geological processes and about the origin of chlorine found in brines and basalts[13,14]. Furthermore, chlorine isotope analysis of perchlorate has been used to identify the source of environmental contamination[15]. "Offline" methods such as the Holt method[16] were for a long time the only way to accomplish such chlorine stable isotope analysis. They rely on a chemical conversion of a compound in sealed glass or metal tubes and complex vacuum lines followed by isotope ratio mass spectrometry (IRMS). Hence, to enable isotope analysis of single compounds, target substances have to be purified beforehand. Afterwards they must be converted into a suitable analyte containing only one chlorine atom such as methyl chloride in the case of the Holt method[16] or CsCl for thermal ionization mass spectrometry[17]. This approach, however, is rather time, labor and cost intensive, requires a large sample amount[16,18] and is therefore prohibitive for compound-specific isotope analysis (CSIA) of organic compounds in trace concentrations. In turn, such chlorine CSIA has recently been made possible by advancing and optimizing instrumentation for online chlorine isotope analysis. Chromatographic separation of a sample is combined with subsequent isotope ratio analysis by a dedicated IRMS[19]. First instrumental solutions for chlorine CSIA were realized by transferring the separated chlorinated compounds in a Helium carrier gas stream directly to an IRMS with dedicated cup configuration[9] or into a quadrupole mass spectrometer (qMS)[20–22]. In a most recent development, chlorine isotope analysis via GC-MC-ICPMS (gas chromatography - multicollector inductively coupled plasma mass spectrometer) has even been realized by converting organic compounds into Cl^+ ions in an inductively coupled plasma and, therefore, offering for the first time an opportunity of universal online chlorine CSIA at very low analyte concentrations (2-3 nmol of Cl)[23,24].

Chlorine CSIA has played a key role in elucidating chlorinated ethene transformation mechanisms in lab studies[25–30] and is at the verge of becoming a method of choice to study the environmental fate of chlorinated hydrocarbons at contaminated sites. Even chlorinated compounds with more complex structures like herbicides are getting within reach. At this point, however, an issue is becoming increasingly important that is crucial for chlorine CSIA on unconverted target compounds and is particularly warranted for comparison of analyses by different instrumental approaches: The need for chlorine isotope reference materials and compound-specific in-house isotope working standards.

As expressed by Equation (1) isotope reference standards - ideally two standards which bracket the isotope values of the samples - are crucial for true isotope measurements[1,22,31,32]. International reference materials are highly valuable, rather expensive and sometimes even available only in limited amounts. Therefore, laboratories are advised to prepare their own in-house reference standards. These standards should be calibrated against the international reference standards[33]. In the case of chlorine two international reference materials are available, ISL-354 (NaCl, $\delta^{37}Cl$ = +0.05 ± 0.03 ‰) and NIST SRM 975a (NaCl, $\delta^{37}Cl$ = +0.2 ± 1.5 ‰)[34,35]. Additionally, Böhlke et al.[36] were able to synthesize and characterize the chlorine isotope standards USGS37 ($KClO_4$, $\delta^{37}Cl$ = +0.90 ± 0.04 ‰), USGS38 ($KClO_4$, $\delta^{37}Cl$ = -87.90 ± 0.24 ‰) and USGS39 ($KClO_4$, $\delta^{37}Cl$ = +0.05 ‰) on the international scale. Unfortunately, most of these standards show very similar values. The one standard that shows a large isotopic shift – USGS38 – is only available in a limited amount. Hence, in-house working standards for daily chlorine isotope analysis are often characterized against only one international reference standard raising an urgent need for a second widely available referencing material with a pronounced isotopic shift (Objective 1).

A second challenge lies in the upcoming opportunity of chlorine CSIA which, however, requires sets of compound-specific working standards that bracket a suitable range of isotope values. For chlorine CSIA these working standards have to be substance-specific since there is no combustion to an analyte gas. According to the IT-Principal (Principal of identical treatment of referencing material and sample) the process of measurement can include isotope fractionating steps. Therefore, for each substance the trueness of analysis has to be validated by using chemically identical standards with a known isotope value, which are subject to the same reaction conditions as the sample[21,37,38]. Hence, our second objective was to create such compound-specific working standards (Objective 2).

Even though it is well established that isotopologues can be separated by physical properties like diffusivity or vapor pressure, the corresponding processes require an extensive number of repetitions. To this end, dedicated instrumentation is needed that is beyond the scope of typical isotope laboratories. Alternatively, because most chemical reactions are accompanied by larger isotopic fractionation than physical processes, chemical reactions can be used as a tool to synthesize standards with a more negative or a more positive isotope value than the starting material. To harvest the isotope fractionation of such a chemical reaction, three strategies may be pursued (see Scheme 1): Strategy 1: a substrate may be converted to a large degree, the reaction may be stopped and the remaining substrate may be purified from the reaction mixture. Strategy 2: a product may be continuously recovered in the presence of a large pool of substrate. Strategy 3: if two products are formed simultaneously, a reaction may be brought to completion and the products may be separated to take advantage of the differences in isotope effects to the parallel products.



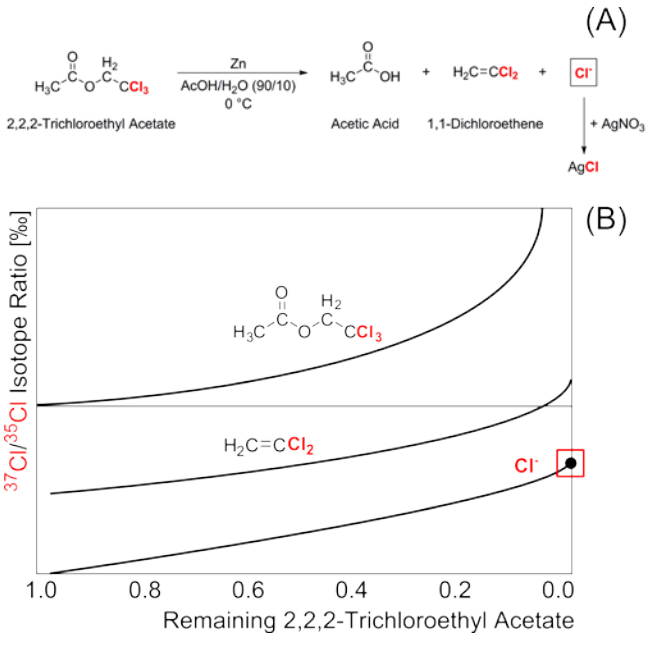
Scheme 1. Strategies which can be applied to generate a standard with a shifted chlorine isotope ratio compared to the starting material.

The first objective of this study was to synthesize and characterize a chloride salt as new chlorine isotope reference standard which can be used as second anchor to characterize in-house working standards in the future. To this end, Strategy 3 of Scheme 1 was pursued to synthesize a chloride in-house reference standard with a negative isotope value. Subsequently, this standard was characterized against the international chlorine reference standards USGS38 and ISL-354.

The second objective of this study was to show that chemical reactions and their corresponding isotope fractionation can be used according to Strategy 1 to generate in-house working standards for chlorine CSIA of specific organic compounds. Since isotope fractionation of micropollutants such as pesticides is receiving increasing attention[39], the herbicides S-Metolachlor and Acetochlor were chosen. These compounds are among the most commonly used herbicides for the protection of plants against weeds in the US agriculture[40]. In the environment they can have toxic effects on living organisms[41]. Thus, studying the environmental fate and the transformation pathways of these herbicides by chlorine CSIA is of particular interest.

EXPERIMENTAL SECTION

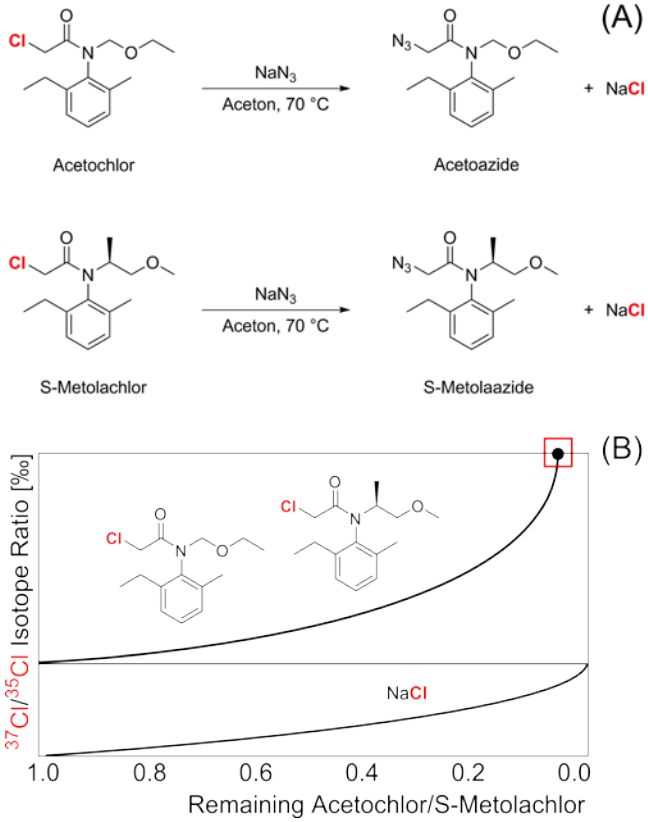
Synthesis of the chlorine isotope reference standard CT16. Following the protocol of Somsak et al.[42], 2,2,2-trichloroethyl acetate (14 ml) was used as starting material. As depicted in Scheme 2A the trichloroethyl group was removed by zinc in 90 % aqueous acetic acid (140 ml) via a reductive elimination process under reflux conditions at 0 °C. After 24 h a silver nitrate solution (350 ml, 17 g/l) was added to precipitate the formed chloride as silver chloride. After filtering, the pure silver chloride (2.61 g) was dried at 40 °C over night in the dark and subsequently stored in a desiccator in the dark.



Scheme 2. (A) Synthesis of the silver chloride reference standard CT16 via reductive elimination of Cl^- and subsequent precipitation with $AgNO_3$. (B) Expected fractionation

and the corresponding strategy to recover chloride with pronounced changes in chlorine isotope values.

Synthesis of the chlorine isotope working standards Aceto2 for Acetochlor and Metola2 for S-Metolachlor. Acetochlor and S-Metolachlor were purchased in their pure forms (Chemos). As such they could be used as primary working standards Aceto1 and Metola1 representing one anchor point of the two-point calibration. To generate working standards with an isotopic shift for a second anchor point, 18 g of the purchased Acetochlor and S-Metolachlor, respectively, and NaN_3 (21.6 g/20.7 g) were dissolved in acetone (500 ml) according to the protocol of Weigl & Wünsch[43]. Like illustrated in Scheme 3A the solution was heated up to 70 °C under reflux. During the reaction chloride was substituted by an azide group. The progress of the reaction was monitored via HPLC analysis. The reactions were stopped when 32.2 % of the initial Acetochlor (approx. after 40 h) and 29.4 % of initial S-Metolachlor (approx. after 94 h) were left. By flushing the solution with N_2 the solvent was evaporated at room temperature. The residue was twice dissolved in diethyl ether (200 ml), the ether phase was washed three times with H_2O (300 ml) and dried over Na_2SO_4. After filtering and evaporating with a rotary evaporator, the unconverted respective chloroacetanilide was purified and recovered from the rest of the reaction mixture via silica column chromatography. The eluent was n-hexane/ethyl acetate (6/1) for acetochlor, (R_f = 0.36) and n-hexane/ethyl acetate (4/1) for S-Metolachlor (R_f = 0.447) respectively. In a last step the eluent was removed by rotary evaporation. 1.43 g of Acetochlor (reddish oil) and 2.53 g S-Metolachlor (yellowish oil) were obtained.



Scheme 3. (A) Synthesis of chlorine isotope working standards of Acetochlor Aceto2 and S-Metolachlor Metola2. (B) Expected fractionation and resultant strategy to recover unreacted Acetochlor/S-Metolachlor with pronounced changes in chlorine isotope values.

Monitoring of the reaction progress of Acetochlor and S-Metolachlor via HPLC. For HPLC analysis 1 ml of reaction solution was sampled and the solvent was evaporated by flushing the sample with N_2. The residue was dissolved in 1 ml acetonitrile and analyzed on a Shimadzu UHPLC-20A system. To this end samples were diluted (1:200) with MilliQ/acetonitrile (80/20). A C18 column (Purospher STAR, RP-18 endcapped (5 µm), LiChroCART 125-2, Merck) was used together with acetonitrile and a KH_2PO_4 buffer (0.1 mM) as eluents. A volume of 5 µl was injected and the oven temperature was set to 40 °C. Separation was accomplished by gradient elution at a flow rate of 0.3 ml/min starting with 40 % acetonitrile and 60 % buffer. For separation of Acetochlor and Acetoazide a linear gradient to 70 % acetonitrile within 33 min was used, whereas S-Metolachlor and S-Metolaazide were separated in a linear gradient to 60 % acetonitrile within 22 min. The respective final conditions were maintained isocratic for 4 min and 3 min, respectively, before a subsequent gradient led back to the initial conditions of 40 % acetonitrile within 1 min and 0.5 min, respectively. Subsequent equilibration was for 5 min (Acetochlor) and 7.5 min (S-Metolachlor). Compound detection took place by UV absorbance at a wavelength of 216 nm for Acetochlor and at 214 nm for S-Metolachlor. Quantification was performed by the software "Lab Solutions".

Conversion of the international reference standard ISL-354 (NaCl) to silver chloride. The conversion of ISL-354 (NaCl (56 mg) dissolved in 50 ml MilliQ) was accomplished by precipitation with 30 ml of silver nitrate solution (20.3 mg/ml). The precipitated silver chloride was washed twice with methanol and once with acetone. Afterwards it was dried at room temperature in the dark.

Conversion of the international reference standard USGS38 ($KClO_4$) to silver chloride. Following the protocol of Böhlke et al.[36] $KClO_4$ (2.5 mg) was filled into quartz glass ampoules which were then evacuated and sealed with an oxygen torch. After heating the ampoules to 720 °C for 20 min in a preheated oven, they were cracked and the Cl^- that was formed from decomposed ClO_4^- was dissolved in 2 ml warm MilliQ water. Silver chloride was precipitated by adding 0.1 ml of silver nitrate solution (83.3 mg/ml). The silver chloride was then washed twice with methanol and once with acetone and dried at room temperature in the dark.

Conversion of silver chloride to methyl chloride. A method for the conversion of silver chloride to methyl chloride was modified from Holt et al.[16]. Silver chloride (300 µg) was weighted into 10 ml headspace vials and flushed for 20 seconds with N_2 gas. Methyl iodide (150 µl) which was filled into 1.5 ml quartz glass inserts was added. Afterwards the vials were closed and tightly crimped with

PTFE coated septa (Carl Roth) and put into the oven at 80 °C for 48 h.

Chlorine isotope analysis via GC-IRMS (Munich). The method for chlorine isotope analysis was adapted from Shouakar-Stash et al.[44]. Measurements were performed on a gas chromatograph (Thermo Scientific, Trace GC Ultra) coupled to an isotope ratio mass spectrometer (Thermo Scientific, Finnegan MAT 253 IRMS) equipped with a direct transfer line so that the MeCl samples were directly transferred from the GC to the IRMS in a He carrier stream. There, the compounds were ionized and fragmented for isotope ratio analysis at the masses m/z of 50/52. To achieve optimal separation, a Vocol column (Supelco, 30 m x 0.25 mm, 1.5 µm film thickness) was used. Samples from the headspace (250 µl) were injected into the GC at a split ratio of 1:50. The GC oven temperature program started at 40 °C (1 min), increased to 100 °C at 30 °C/min and was held for 2 min. MeCl reference gas pulses were injected via a dual inlet system at the beginning and at the end of each measurement as described in Bernstein et al.[22]. Two-point calibrations were performed with the international reference standards ISL-354 ($\delta^{37}Cl$ = +0.05 ‰)[34] and USGS38 ($\delta^{37}Cl$ = -87.90 ‰)[36] to convert measurements to $\delta^{37}Cl$ values relative to Standard Mean Ocean Chloride (SMOC).

Chlorine isotope analysis via GC-MC-ICPMS (Leipzig). Measurements were performed according to the protocols described in Horst et al. 2017[24] and Renpenning et al. 2018[23]. Samples were separated using a gas chromatograph (Thermo Scientific, Trace 1310) equipped with a Zebron ZB-1 column (Phenomenex Inc., 60 m x 0.32 mm, 1 µm film thickness). A heated transfer line coupled the GC to a multi-collector inductively coupled plasma mass spectrometer (MC-ICPMS, Thermo Fisher Scientific, Neptune). For analysis of methyl chloride 80 µl of gaseous sample were manually injected into a split/splitless injector at a temperature of 280 °C. For achieving chromatographic separation of methyl chloride and methyl iodide the GC started at 30 °C (8 min) followed by a gradient of 10 °C/min to 100 °C. The transfer line was kept at 160 °C. A constant column flow of 2 ml/min with a split ratio of 1:10 was applied. For the analysis of Acetochlor and S-Metolachlor the pure substances were diluted in acetone to a final concentration of 2 ppm. Three microliters of liquid sample were manually injected into the same injector kept at a temperature of 250 °C. The GC started at a temperature of 100 °C, after 3 min it increased to 240 °C at 20 °C/min, followed by an increase to 300 °C at 5 °C/min and hold for 5 min. The transfer line had a temperature of 280 °C. A constant column flow of 2 ml/min with a split ratio of 1:10 was applied. In-house referencing standards "TCE-2" (-2.54 ± 0.13 ‰) and "MeCl" (4.49 ± 0.10 ‰) were calibrated against methyl chloride from ISL-354 and USGS38 and subsequently used for preliminary characterization of Acetochlor and S-Metolachlor. In addition, further compounds (Acetochlor, S-Metolachlor, Atrazine) were purchased and calibrated the same way. For results see Table S1 (SI).

RESULTS AND DISCUSSION

A new chlorine isotope reference standard on the international scale. Reductive dehalogenation of 2,2,2-trichloroethyl acetate by zinc powder produced 1,1-dichloroethene (not analyzed) and chloride, which could be precipitated and isolated as AgCl. The pure silver chloride as candidate for a new in-house reference standard was given the name "CT16". For chlorine isotope analysis it was subsequently converted to methyl chloride in order to facilitate isotopic characterization by GC-MC-ICPMS and GC-IRMS against international reference standards treated in the same way.

Figure 1 shows that the newly synthesized CT16 in-house chlorine isotope reference standard was adequately bracketed by the international reference standards ISL-354 and USGS38. A first characterization of CT16 via GC-IRMS in September 2017 resulted in a value of $\delta^{37}Cl_{CT16}$ = -26.82 ± 0.17 ‰ (see Figure 1A). These measurements were repeated in February 2018 yielding a value of $\delta^{37}Cl_{CT16}$ = -26.88 ± 0.28 ‰ that was identical to the first one within the analytical uncertainty (see Figure 1B). In a third approach the CT16 was characterized via GC-MC-ICPMS giving even more precise values ($\delta^{37}Cl_{CT16}$ = -26.75 ± 0.08 ‰) which were in accordance with the GC-IRMS results (see Figure 1C). Consequently, the mean value over all measurements, $\delta^{37}Cl_{CT16}$ = -26.82 ± 0.18 ‰ (n = 16), is considered as "true" consensus value. As intended, this value shows a relatively large shift when compared to most international chlorine isotope reference standards which center on an isotope value of 0 ‰[34-36].

This strong negative value can be explained by the isotope effect of the reaction. During the reductive elimination depicted in Scheme 2A, chlorine isotope fractionation is expected to take place according to Scheme 2B. Bonds containing heavy isotopes are slightly more stable than bonds containing light isotopes so that bonds with light isotopes break faster[2,25]. Consequently, ^{35}Cl is preferentially cleaved off from 2,2,2-trichloroethyl acetate meaning that the produced chloride in solution is expected to contain less ^{37}Cl per ^{35}Cl. This leads to isotope values that are strongly negative compared to the formed 1,1-dichloroethene, compared to the original substrate and also compared to most available reference materials to date.

This opportunity to create materials with negative chlorine isotope values clearly represents a great advantage in future characterization of chlorine isotope standards: in-house working standards can be calibrated against two different reference standards – against one reference standard with an isotope value close to 0 ‰ and against CT16. Since a range from 0 ‰ to -26.82 ‰ is covered, results for the characterization of in-house chlorine working standard will become more accurate which will consequently also increase the precision and trueness of daily chlorine isotope measurements of samples[1,22,31].

Candidate compounds for compound-specific chlorine isotope working standards. Reactions of Aceto-

chlor and S-Metolachlor with sodium azide were stopped when app. 70 % of the substrates were converted to sodium chloride and acetoazide and metolaazide, respectively. The remaining substrates were named “Aceto2” and “Metola2”. Together with the original substances, which were named “Aceto1” and “Metola1”, the isolated Aceto2 and Metola2 were measured via GC-MC-ICPMS.

Figure 2 shows that the synthesized working standards Aceto2 and Metola2 exhibit significantly more positive chlorine isotope values than the initial substances Aceto1 and Metola1. For Acetochlor, the initial substance Aceto1 was characterized to have an isotope value of $\delta^{37}Cl_{Aceto1}$ = 0.29 ± 0.29 ‰ tentatively determined by GC-MC-ICPMS. The synthesized working standard Aceto2 shows an isotope value of $\delta^{37}Cl_{Aceto2}$ = 18.54 ± 0.20 ‰ corresponding to an isotopic shift of app. 18 ‰ (see Figure 2A). Measurements of S-Metolachlor resulted in an isotopic shift of app. 9 ‰. By the same GC-MC-ICPMS analysis Metola1, the initial substance, was attributed a chlorine isotope value of $\delta^{37}Cl_{Metola1}$ = -4.28 ± 0.17 ‰ and the synthesized working standard, Metola2, of $\delta^{37}Cl_{Metola2}$ = 5.12 ± 0.27 ‰ (see Figure 2B).

The change in chlorine isotope values for each of the two substances happened due to the isotope effect of the underlying second order nucleophilic chemical substitution reaction (S_N2, Scheme 3A). As illustrated in Scheme 3B, owing to the leaving group isotope effect associated with chloride substitution, the remaining substrate gets enriched in heavy relative to light chlorine isotopes leading to a more positive chlorine isotope value compared to the chlorine isotope value of the original substrate before the start of the reaction.

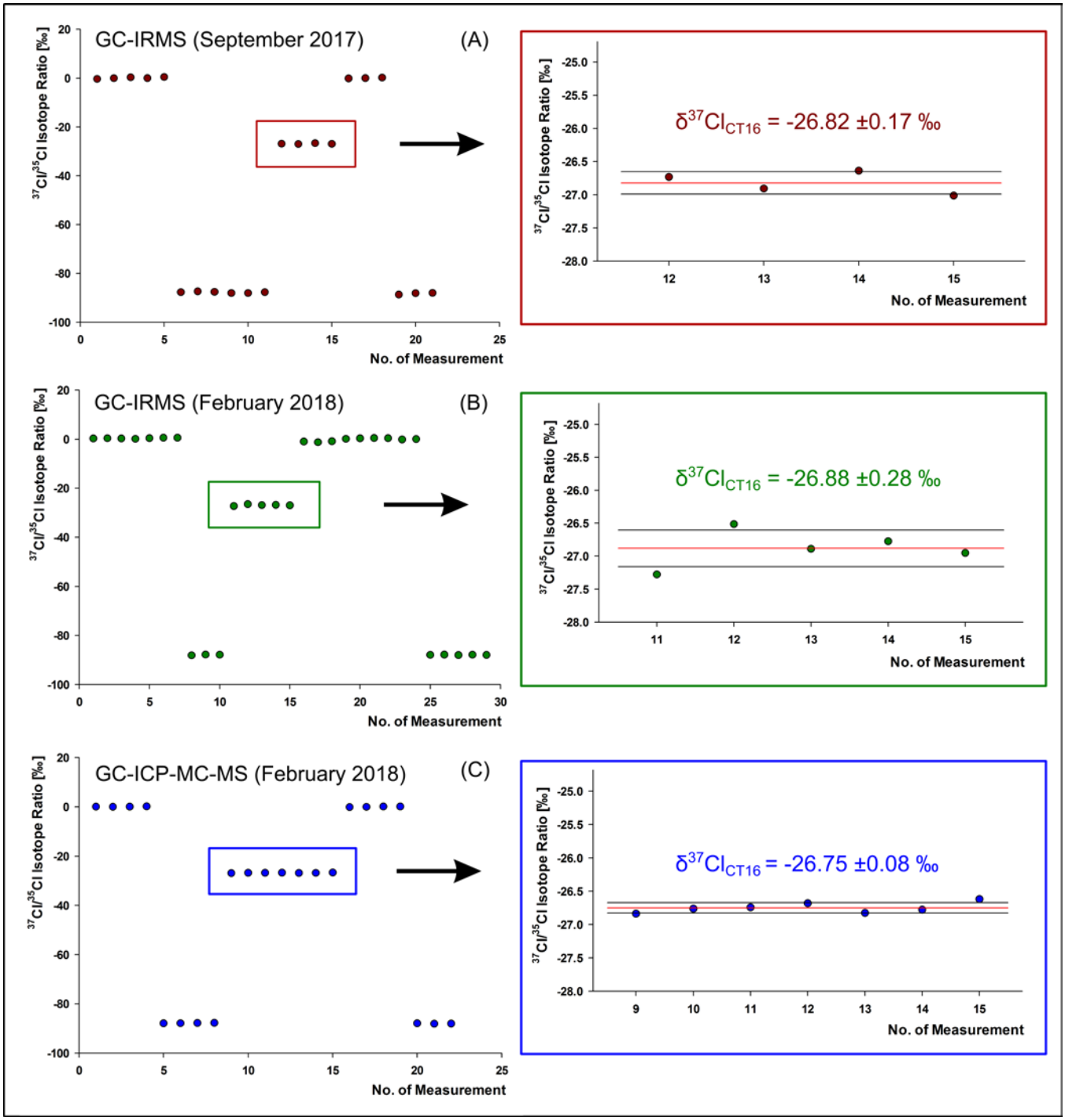


Figure 1. Characterization of the synthesized silver chloride reference standard CT16 against the international reference standards ISL-354 ($\delta^{37}Cl$ = +0.05 ‰) and USGS38 ($\delta^{37}Cl$ = -87.90 ‰). (A/B) CT16 measured via GC-IRMS in Munich at two different time points in (A) September 2017 and (B) February 2018, (C) CT16 measured via GC-MC-ICPMS in Leipzig in February 2018. (The mean is given as value and as red line, while standard deviations are given as values and as black lines).

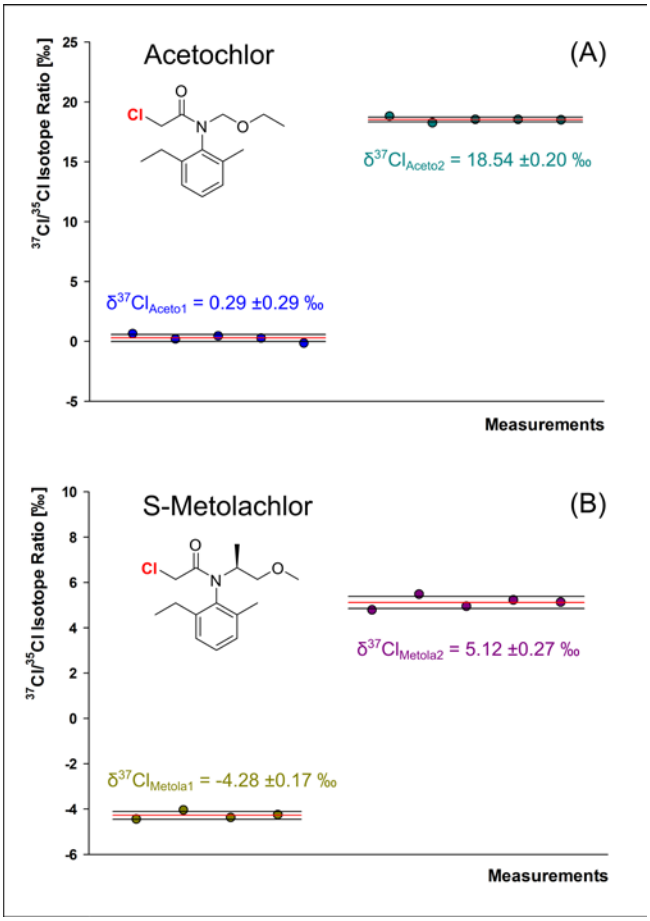


Figure 2. Characterization of (A) the Acetochlor working standards Aceto1 and Aceto2 and (B) the S-Metolachlor working standards Metola1 and Metola2. (The mean is given as value and as red line, while standard deviations are given as values and as black lines).

These results illustrate that organic chemistry can be used to generate substance-specific working standards with pronounced shifts in chlorine isotope values. Thus, working standards for other chlorinated complex organic compounds can be generated in the future so that stable chlorine isotope analysis of these compounds will help to further illuminate their transformation pathways.

CONCLUSION

Five new standards, the reference standard CT16 and the working standards Aceto1 / Aceto2 and Metola1 / Metola2, for stable chlorine isotope analysis were synthesized. In particular, the synthesis route to silver chloride (CT16) provides an opportunity to generate much-needed in-house reference standards of chloride. The possibility to use two reference standards which differ in their chlorine isotope value will optimize future characterization results of chlorine working standards. More accurate in-house working standards will in turn optimize the precision and trueness of daily chlorine isotope measurements. In addition, the synthesis of the working standards for Acetochlor (Aceto1 and Aceto2) and S-Metolachlor (Metola1 and Metola2) showed that organic synthesis can generate substance-specific isotope working standards also of more complex chlorinated organic compounds. These working standards become even more important as GC-qMS methods for stable chlorine isotope analysis of Acetochlor and S-Metolachlor were recently developed by Ponsin et al. (in preparation)[45]. However, two of the working standards show a chlorine isotope value larger than 0 ‰ ($\delta^{37}Cl_{Aceto2}$ = 18.54 ± 0.20 ‰ and $\delta^{37}Cl_{Metola2}$ = 5.12 ± 0.27 ‰). Therefore, future work targeting the synthesis of a chloride reference standard with a more positive chlorine isotope value would optimize the characterization process of in-house working standards even further. In a next step our work provides the opportunity to recalibrate the compound-specific in-house working standards, which were used so far for stable chlorine isotope analysis. Initially they were characterized against only one reference standard. Now we are able to recalibrate them using a two-point calibration. The ongoing development of new calibration standards together with the advancement of stable chlorine isotope analysis now offers a suite of accurate methods for chlorine isotope analysis (offline DI-IRMS, online GC-MS and GC-IRMS, offline and online MC-ICPMS). By using these synergistic effects, the development of stable isotope analysis of chlorine can be further accelerated which will open up new perspectives to study environmental contaminants and to characterize commercial products in the future.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

3 Schemes illustrating the workflow of isotopic characterization, Table showing additional preliminary results of chlorine working standards for other semi-volatile chlorinated compounds (PDF)

AUTHOR INFORMATION

Corresponding Author

* Phone: +49 89/2180-78231. E-mail: m.elsner@tum.de

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENT

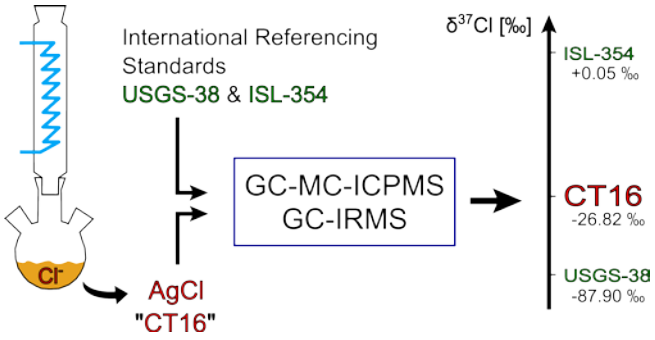
This work was supported by the Swiss National Science Foundation (SNSF, Grant CRSII2_141805) and the German-Israeli Foundation for Scientific Research and Development (GIF, Grant I-251-307.4-2013). We are grateful to the Centre for Chemical Microscopy (Pro-VIS) at the Helmholtz Centre

for Environmental Research supported by European Regional Development Funds (EFRE – Europe Funds Saxony) for using the MC-ICPMS at their analytical facilities.

REFERENCES

(1) Coplen, T. B.; Böhlke, J. K.; De Bievre, P.; Ding, T.; Holden, N. E.; Hopple, J. A.; Krouse, H. R.; Lamberty, A.; Peiser, H. S.; Revesz, K.; Rieder, S. E.; Rosman, K. J. R.; Roth, E.; Taylor, P. D. P.; Vocke, R. D.; Xiao, Y. K. *Pure Appl. Chem.* **2002**, *74*, 1987-2017.
(2) Elsner, M. *J. Environ. Monit.* **2010**, *12*, 2005-2031.
(3) Coplen, T. B. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 2538-2560.
(4) Hunkeler, D.; Meckenstock, R. U.; Sherwood Lollar, B.; Schmidt, T.; Wilson, J.; Schmidt, T.; Wilson, J., A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA); PA 600/R-08/148 | December 2008 | www.epa.gov/ada; US EPA: Oklahoma, USA, 2008.
(5) Hakenbeck, S.; McManus, E.; Geisler, H.; Grupe, G.; O'Connell, T. *Am. J. Phys. Anthropol.* **2010**, *143*, 235-249.
(6) Rossmann, A. *Food Rev. Int.* **2001**, *17*, 347-381.
(7) Desage, M.; Guilluy, R.; Brazier, J. L.; Chaudron, H.; Girard, J.; Cherpin, H.; Jumeau, J. *Anal. Chim. Acta* **1991**, *247*, 249-254.
(8) Cawley, A. T.; Flenker, U. *J. Mass Spectrom.* **2008**, *43*, 854-864.
(9) Schmidt, T. C.; Zwank, L.; Elsner, M.; Berg, M.; Meckenstock, R. U.; Haderlein, S. B. *Anal. Bioanal. Chem.* **2004**, *378*, 283-300.
(10) Hofstetter, T. B.; Berg, M. *TrAC, Trends Anal. Chem.* **2011**, *30*, 618-627.
(11) Aelion, C. M.; Hohener, P.; Hunkeler, D.; Aravena, R.; CRC Press, 2009.
(12) Fenner, K.; Canonica, S.; Wackett, L. P.; Elsner, M. *Science* **2013**, *341*, 752-758.
(13) Magenheim, A. J.; Spivack, A. J.; Michael, P. J.; Gieskes, J. M. *Earth Planet. Sci. Lett.* **1995**, *131*, 427-432.
(14) Bonifacie, M.; Jendrzejewski, N.; Agrinier, P.; Humler, E.; Coleman, M.; Javoy, M. *Science* **2008**, *319*, 1518-1520.
(15) Böhlke, J. K.; Sturchio, N. C.; Gu, B.; Horita, J.; Brown, G. M.; Jackson, W. A.; Batista, J.; Hatzinger, P. B. *Anal. Chem.* **2005**, *77*, 7838-7842.
(16) Holt, B. D.; Sturchio, N. C.; Abrajano, T. A.; Heraty, L. J. *Anal. Chem.* **1997**, *69*, 2727-2733.
(17) Xiao, Y.-K.; Zhang, C.-G. *Int. J. Mass Spectrom. Ion Processes* **1992**, *116*, 183-192.
(18) de Groot, P. A. In *Handbook of Stable Isotope Analytical Techniques, Vol. 2* de Groot, P. A., Ed.; Elsevier: Amsterdam, 2009, pp 229-269.
(19) Shouakar-Stash, O.; Drimmie, R. J.; Frape, S. K. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 121-127.
(20) Sakaguchi-Soder, K.; Jager, J.; Grund, H.; Matthaus, F.; Schuth, C. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3077-3084.
(21) Aeppli, C.; Holmstrand, H.; Andersson, P.; Gustafsson, O. *Anal. Chem.* **2010**, *82*, 420-426.
(22) Bernstein, A.; Shouakar-Stash, O.; Ebert, K.; Laskov, C.; Hunkeler, D.; Jeannottat, S.; Sakaguchi-Soder, K.; Laaks, J.; Jochmann, M. A.; Cretnik, S.; Jager, J.; Haderlein, S. B.; Schmidt, T. C.; Aravena, R.; Elsner, M. *Anal. Chem.* **2011**, *83*, 7624-7634.
(23) Renpenning, J.; Horst, A.; Schmidt, M.; Gehre, M. *J. Anal. At. Spectrom.* **2018**, *33*, 314-321.
(24) Horst, A.; Renpenning, J.; Richnow, H.-H.; Gehre, M. *Anal. Chem.* **2017**, *89*, 9131-9138.
(25) Cretnik, S.; Bernstein, A.; Shouakar-Stash, O.; Löffler, F.; Elsner, M. *Molecules* **2014**, *19*, 6450-6473.
(26) Wiegert, C.; Mandalakis, M.; Knowles, T.; Polymenakou, P. N.; Aeppli, C.; Macháčková, J.; Holmstrand, H.; Evershed, R. P.; Pancost, R. D.; Gustafsson, Ö. *Environ. Sci. Technol.* **2013**, *47*, 6449-6456.
(27) Badin, A.; Buttet, G.; Maillard, J.; Holliger, C.; Hunkeler, D. *Environ. Sci. Technol.* **2014**, *48*, 9179-9186.
(28) Renpenning, J.; Keller, S.; Cretnik, S.; Shouakar-Stash, O.; Elsner, M.; Schubert, T.; Nijenhuis, I. *Environ. Sci. Technol.* **2014**, *48*, 11837–11845.
(29) Abe, Y.; Aravena, R.; Zopfi, J.; Shouakar-Stash, O.; Cox, E.; Roberts, J. D.; Hunkeler, D. *Environ. Sci. Technol.* **2009**, *43*, 101-107.
(30) Heckel, B.; McNeill, K.; Elsner, M. *ACS Catal.* **2018**, *8*, 3054-3066.
(31) Debajyoti, P.; Grzegorz, S.; István, F. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3006-3014.
(32) Heckel, B.; Rodríguez-Fernández, D.; Torrentó, C.; Meyer, A.; Palau, J.; Domènech, C.; Rosell, M.; Soler, A.; Hunkeler, D.; Elsner, M. *Anal. Chem.* **2017**, *89*, 3411-3420.
(33) Hut, G. **1987**.
(34) Xiao, Y.; Yinming, Z.; Qingzhong, W.; Haizhen, W.; Weiguo, L.; Eastoe, C. *Chem. Geol.* **2002**, *182*, 655-661.
(35) Gröning, M. In *Handbook of Stable Isotope Analytical Techniques: Volume I*; Elsevier, 2004, pp 874-906.
(36) Böhlke, J.; Mroczkowski, S. J.; Sturchio, N. C.; Heraty, L. J.; Richman, K. W.; Sullivan, D. B.; Griffith, K. N.; Gu, B.; Hatzinger, P. B. *Rapid Commun. Mass Spectrom.* **2016**.
(37) Elsner, M.; Jochmann, M. A.; Hofstetter, T. B.; Hunkeler, D.; Bernstein, A.; Schmidt, T. C.; Schimmelmann, A. *Anal. Bioanal. Chem.* **2012**, *403*, 2471-2491.
(38) Werner, R. A.; Brand, W. A. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 501-519.
(39) Elsner, M.; Imfeld, G. *Curr. Opin. Biotechnol.* **2016**, *41*, 60-72.
(40) EPA. *US Environmental Protection Agency, Washington (DC)* **2017**.
(41) Dearfield, K. L.; McCarroll, N. E.; Protzel, A.; Stack, H. F.; Jackson, M. A.; Waters, M. D. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **1999**, *443*, 183-221.
(42) Somsák, L.; Czifrák, K.; Veres, E. *Tetrahedron Lett.* **2004**, *45*, 9095-9097.
(43) Weigl, M.; Wünsch, B. *Tetrahedron* **2002**, *58*, 1173-1183.
(44) Shouakar-Stash, O.; Drimmie, R. J.; Zhang, M.; Frape, S. K. *Appl. Geochem.* **2006**, *21*, 766-781.
(45) Ponsin, V.; Torrentó, C.; Lihl, C.; Elsner, M.; Hunkeler, D. **in preparation**.

Graphical Abstract:


International Referencing
Standards
USGS-38 & ISL-354
GC-MC-ICPMS
GC-IRMS
Cl-
AgCl
"CT16"
δ37Cl [‰]
ISL-354
+0.05 ‰
CT16
-26.82 ‰
USGS-38
-87.90 ‰