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Carbon and chlorine isotope analysis to identify abiotic degradation pathways of 1,1,1-trichloroethane

Jordi Palau,[†][*] Orfan Shouakar-Stash,[§] and Daniel Hunkeler[†]

[†]Centre for Hydrogeology and Geothermics, University of Neuchâtel, Neuchâtel, Switzerland.

[§]Department of Earth and Environmental Sciences, University of Waterloo, Waterloo, Canada.

ABSTRACT

This study investigates dual C-Cl isotope fractionation during 1,1,1-TCA transformation by heat-activated persulfate (PS), hydrolysis/dehydrohalogenation (HY/DH) and Fe(0). Compound-specific chlorine isotope analysis of 1,1,1-TCA was performed for the first time, and transformation-associated isotope fractionation ε_{bulk}^C and ε_{bulk}^{Cl} were: -4.0±0.2‰ and no chlorine isotope fractionation with PS, -1.6±0.2‰ and -4.7±0.1‰ for HY/DH, -7.8±0.4‰ and -5.2±0.2‰ with Fe(0). Distinctly different dual isotope slopes ($\Delta\delta^{13}C/\Delta\delta^{37}Cl$): ∞ with PS, 0.33±0.04 for HY/DH and 1.5±0.1 with Fe(0) highlight the potential of this approach to identify abiotic degradation pathways of 1,1,1-TCA in the field. The trend observed with PS agreed with a C-H bond oxidation mechanism in the first reaction step. For HY/DH and Fe(0) pathways, different slopes were obtained although both pathways involve cleavage of a C-Cl bond in their initial reaction step. In contrast to the expected larger primary carbon isotope effects relative to chlorine for C-Cl bond cleavage, $\varepsilon_{bulk}^C < \varepsilon_{bulk}^{Cl}$ was observed for HY/DH and in a similar range for reduction by Fe(0), suggesting the contribution of secondary chlorine isotope effects. Therefore, different magnitude of secondary chlorine isotope effects could at least be partly responsible for the distinct slopes between HY/DH and Fe(0) pathways. Following this dual isotope approach abiotic transformation processes can unambiguously be identified and quantified.

INTRODUCTION

Chlorinated ethanes are common groundwater contaminants and 1,1,1-trichloroethane (1,1,1-TCA) has been found in at least half of the 1,662 National Priorities List sites identified by the U. S. Environmental Protection Agency (USEPA).[1, 2] 1,1,1-TCA has been widely used as solvent and its presence in the subsurface mainly results from industrial activity. In groundwater, 1,1,1-TCA may be transformed via multiple biotic and abiotic reaction pathways,[3, 4] making it challenging to identify degradation processes. Identifying processes is further complicated by the possibility of having the same products from different precursors. Abiotic 1,1,1-TCA degradation may yield daughter products such as 1,1-dichloroethene (1,1-DCE), ethene or ethane that can be formed from different precursors in sites impacted by a mixture of chlorinated compounds.[4, 5] In this case, parent-daughter relationships based solely on concentration data may lead to erroneous interpretations. Therefore, development of innovative methods for the elucidation of 1,1,1-TCA abiotic degradation pathways in the environment as well as in engineered systems is warranted.

Reported sequential anaerobic biodegradation of 1,1,1-TCA generally stalls at the stage of chloroethane (CA),[6-8] a persistent groundwater contaminant considered as priority pollutant by the USEPA.[9] Therefore, abiotic reactions of 1,1,1-TCA yielding non-chlorinated products are of special interest. In groundwater, 1,1,1-TCA is abiotically dechlorinated to acetate (Ac) and 1,1-DCE by hydrolysis (nucleophilic substitution) and dehydrohalogenation (β-elimination), respectively.[10, 11] Hydrolysis and dehydrohalogenation (HY/DH) may proceed either via two parallel reactions starting from the substrate (S_N2 vs. E2) or via a common pathway through formation of a carbocation intermediate (Scheme 1a).[12] However, according to the results from previous reaction studies[11-13] the second mechanistic scenario seems more likely. In anoxic conditions, 1,1,1-TCA can also undergo metal-catalyzed reduction by natural reductants such as iron sulfides.[14, 15] Transformation of 1,1,1-TCA by zero-valent metals and

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72 bimetallic reductants has been investigated to evaluate their potential application to in situ

73 treatment techniques such as nanoparticles injection or permeable reactive barriers.[16-20] For

74 1,1,1-TCA reductive dechlorination by zero-valent iron, cleavage of a C-Cl bond by

75 dissociative single electron transfer (SET) and formation of 1,1-dichoroethyl radical

76 intermediate has been proposed as the first reaction step (Scheme 1b), leading to the

77 production of 1,1-dichloroethane (1,1-DCA, via hydrogenolysis), ethene/ethane (α-

78 elimination) and C_4 compounds (coupling).[16, 18] Furthermore, recently 1,1,1-TCA degradation

79 by activated persulfate (PS) using different methods such as base[21] and heat[22-25] activation

80 was demonstrated. Thermal decomposition of PS generates reactive oxygen species[25] and, for

81 reaction with 1,1,1-TCA, transformation via H abstraction[25] has been postulated (Scheme 1c).

82 Previous studies[23, 24, 26] suggested that thermally activated PS could be used for in situ

83 chemical treatment of volatile organic compounds, however, a current limitation is the scarce

84 knowledge of PS reaction pathways in the subsurface.[27]

85 Due to the susceptibility of 1,1,1-TCA to abiotic transformations, two of the three pathways

86 investigated in this study (Scheme 1) may occur simultaneously in the aquifer. In anoxic

87 conditions, reduction of 1,1,1-TCA by natural reductants[15] or engineered Fe(0)-systems[28] can

88 occur in addition to HY/DH. On the other hand, the rate of HY/DH will increase significantly

89 during treatment of 1,1,1-TCA by heat-activated PS due to the raise of water temperature.[10] In

90 fact, thermal enhancement of HY/DH has recently been proposed for in situ remediation of

91 1,1,1-TCA.[29]

92 Compound-specific isotope analysis (CSIA) is an innovative tool to investigate reaction

93 mechanisms of organic contaminants and their degradation pathways in the environment

94 because the extent of isotope fractionation (ε_{bulk}) during compound transformation is highly

95 reaction-specific.[30-34] For organic contaminants, transformation-induced isotope fractionation

96 is generally larger than the one related to phase transfer processes such as sorption or

97   volatilization.[35] While isotope fractionation of one element alone (e.g. ε_{bulk}^{C}) could provide

98   pathway distinction in laboratory experiments,[36] it is not possible under field conditions.

99   Here, contaminant concentration changes related to processes other than its transformation

100   (such as sorption or dilution) cannot be excluded, preventing accurate calculation of ε_{bulk}

101   values. Therefore, a dual isotope approach is necessary to differentiate contaminant

102   degradation pathways in the field.[37] Recent development of analytical methods for online Cl-

103   CSIA, either by continuous flow gas chromatography isotope ratio mass spectrometry (GC-

104   IRMS)[38] or GC-quadrupole mass spectrometry (GC-qMS),[37, 39-41] has simplified the

105   measurement of chlorine isotope ratios in chlorinated compounds.[42, 43] These new methods

106   open new possibilities for a dual C-Cl isotope approach, which has not been applied to 1,1,1-

107   TCA yet.

108   During the course of a reaction, combined changes in isotope ratios (e.g. $\Delta\delta^{13}C$ vs. $\Delta\delta^{37}Cl$) for

109   a given reactant generally yields a linear trend in a dual element isotope plot.[44, 45] The dual

110   element isotope slope ($\Lambda = \Delta\delta^{13}C/\Delta\delta^{37}Cl$) reflects isotope effects of both elements[30] and, thus,

111   different slopes may be expected for distinct transformation mechanisms involving different

112   bonds with distinct elements. Following this approach, dual isotope slopes observed in the

113   field can be compared to the slopes determined in laboratory experiments to identify

114   degradation pathways. For a given compound, knowledge whether different abiotic reactions

115   lead to characteristic slopes in dual C-Cl isotope plots is still very limited for chlorinated

116   ethenes[46-48] and, to our knowledge, currently non-existent for chlorinated ethanes. This

117   knowledge is a prerequisite for the application of a dual isotope approach to in situ

118   contaminant studies.

119   In addition to the dual isotope approach, evaluation of underlying kinetic isotope effects (i.e.

120   KIE = ^{l}k / ^{h}k, where ^{l}k and ^{h}k are the reaction rates of molecules containing the light and

121   heavy isotopes, respectively) also yields insight into the reaction mechanisms. KIEs derived

122 from ε_{bulk} values, so called *apparent* kinetic isotope effects (AKIEs),[49] may then be compared

123 to theoretical or typical intrinsic KIEs for a given reaction. However, reaction mechanisms

124 sometimes cannot be clearly identified because experimental AKIEs may be smaller than the

125 intrinsic KIE if the latter is masked due to non-fractionating rate limiting steps (such as mass

126 transfer through the cell membrane or transport to the mineral reactive surfaces).[30] Therefore,

127 increasing attention is paid to the dual isotope approach for improved differentiation of

128 reaction mechanisms.[44, 45] A significant advantage of this approach is that Λ often remains

129 constant regardless of masking effects.[50, 51] The reason is that rate limiting preceding steps are

130 generally non-fractionating so that both elements are masked to the same extent.[49] In this

131 case, by taking the ratio of the isotope shift for the two elements (e.g., $\Delta\delta^{13}C/\Delta\delta^{37}Cl$) masking

132 effects cancel out.

133 The main aims of this study were i) to assess the potential of the dual C-Cl isotope approach

134 for identifying abiotic degradation pathways of 1,1,1-TCA and ii) to explore the underlying

135 reaction mechanisms based on resultant dual isotope slopes and estimated ^{13}C and ^{37}Cl-

136 AKIEs. We investigated dual element isotope fractionation of 1,1,1-TCA during HY/DH,

137 reduction by micro-sized Fe(0) and reaction with heat-activated PS in laboratory batch

138 experiments. For the first time, isotope fractionation ε_{bulk}^{Cl} values (for all reactions) and ε_{bulk}^{C}

139 (for HY/DH and reaction with heat-activated PS) were determined. In addition to isotope

140 fractionation in the substrate, carbon isotope ratios of 1,1,1-TCA reaction products were also

141 evaluated.

142

143 **MATERIALS AND METHODS**

144 **Experimental set up**

145 All batch experiments were conducted in duplicate and reaction vials and controls were

146 prepared at an initial 1,1,1-TCA concentration of 0.25 mM using a pure 1,1,1-TCA carbon

147   isotopic working standard ($\delta^{13}C_0$ = -26.3±0.1‰, ± standard deviation (1σ), number of

148   measurements (n) = 5). A list of chemicals and additional experiment details is available in

149   the supporting information (SI).

150   *Batch experiments with PS*. Reaction of 1,1,1-TCA with heat-activated PS (PS/1,1,1-TCA

151   molar ratio of 90/1) was performed under controlled temperature (50 °C) using a thermostatic

152   water bath. Reaction vials containing 42 mL of aqueous solution and a minimal headspace (1

153   mL) were stirred at 200 rpm throughout the experiment. Control vials without PS were carried

154   out in parallel.

155   *HY/DH batch experiments*. Reaction of 1,1,1-TCA in water was conducted at 50 °C in order

156   to increase its reaction rate relative to the rate at ambient temperature (i.e. half-lives ($t_{1/2}$) from

157   1.1 years at 25 °C to 3.6 days at 55 ºC).[10, 11] Reddy et al.[52] did not observe significant

158   variation for chlorine isotope fractionation associated with alkaline dehydrochlorination of

159   1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) in a range of temperatures from 52 to

160   72 °C, revealing no observable temperature dependence. Reaction glass bottles contained 115

161   mL of aqueous solution and 6 mL of headspace to avoid cracking of the bottles in the

162   thermostatic water bath. Control bottles were prepared like the reactors and stored at 4 °C.

163   *Batch experiments with Fe(0)*. Micro-sized iron powder (< 212 μm) was pre-cleaned with acid

164   as described in previous studies.[16, 36] Reactors containing 1 g of Fe(0), 112 mL of aqueous

165   solution and 6 mL of headspace were agitated on an orbital shaker at 200 rpm throughout the

166   course of reaction in order to enable rapid mass transfer of dissolved 1,1,1-TCA to the gas and

167   solid phases. Control bottles without Fe(0) were prepared, sampled and stored in the same

168   way as the reactors.

169   **Concentration and isotopic analysis**

170   <u>Concentration measurements</u>

171 Detailed descriptions of analytical methods as well as details on reaction kinetics calculation

172 are available in the SI. Briefly, liquid samples were collected for the analysis of 1,1,1-TCA,

173 1,1-DCE and 1,1-DCA and measured by GC-MS. For the experiments with Fe(0), gas

174 samples from reactors headspace were collected for the analysis of ethene and ethane and

175 measured by GC-FID. The average concentration of 1,1,1-TCA in the controls remained equal

176 to the initial concentration within the uncertainty (see SI), which indicates that compound

177 losses through the valves and caps during reactors shaking and samples preservation were

178 insignificant. Finally, concentrations of Ac and Cl⁻ were determined by ion chromatography.

179 <u>Carbon and chlorine isotopes analysis</u>

180 Carbon isotope ratios (i.e. $^{13}C/^{12}C$) of 1,1,1-TCA and daughter products (1,1-DCE, 1,1-DCA,

181 ethene and ethane) were determined by GC-IRMS (see details in the SI). Chlorine isotope

182 ratios (i.e. $^{37}Cl/^{35}Cl$) of 1,1,1-TCA were measured by GC-qMS based on the two most

183 abundant fragment ions (97, 99 m/z). These ions correspond to an isotopologue pair

184 ($[^{35}Cl_2{}^{12}C_2{}^{1}H_3]^+$ and $[^{35}Cl^{37}Cl^{12}C_2{}^{1}H_3]^+$, respectively) that differ by one heavy chlorine

185 isotope. The isotope ratio was obtained from the ratio of these isotopologues using eq. 1,[53]

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$$R = \frac{^{37}Cl}{^{35}Cl} = \frac{^{37}p}{^{35}p} = \frac{k}{(n-k+1)} \cdot \frac{^{37}Cl_{(k)}{}^{35}Cl_{(n-k)}}{^{37}Cl_{(k-1)}{}^{35}Cl_{(n-k+1)}} = \frac{1}{2} \cdot \frac{^{99}I}{^{97}I} \qquad (1)$$

188

189 where ^{37}p and ^{35}p are the probabilities of encountering ^{37}Cl and ^{35}Cl, n is the number of Cl

190 atoms, k is the number of ^{37}Cl isotopes, $^{37}Cl_{(k)}{}^{35}Cl_{(n-k)}$ and $^{37}Cl_{(k-1)}{}^{35}Cl_{(n-k+1)}$ represent

191 the isotopologues containing k and (k-1) heavy isotopes, respectively, and "I" indicates the

192 ion peak intensities. Isotope ratios of individual compounds were reported using the delta

193 notation (eq. 2),

194

195     $\delta\ ^hE_{sample} = \frac{R(^hE/\ ^lE)_{sample}}{R(^hE/\ ^lE)_{standard}} - 1$ (2)

196

197   where R is the isotope ratio of heavy (^hE) and light (^lE) isotopes of element E (e.g., $^{13}C/^{12}C$

198   and $^{37}Cl/^{35}Cl$). For chlorine, the raw $\delta^{37}Cl$ values were obtained by referencing against an

199   external laboratory 1,1,1-TCA standard according to eq. 2. This standard was dissolved in

200   water and measured like the samples interspersed in the same sequence.[41] Samples and

201   standards were diluted to a similar concentration and each of them was measured ten times.

202   Further details about samples and standards analysis scheme as well as raw $\delta^{37}Cl$ values (two-

203   point) calibration to the standard mean ocean chloride (SMOC) scale are available in the SI.

204   Precision (1σ) of the analysis was 0.3‰ (except for ethane, 0.5‰) for $\delta^{13}C$ and 0.4‰ for

205     $\delta^{37}Cl$.

206   **Evaluation of 1,1,1-TCA isotope fractionation (ε_{bulk}).**

207   The ε_{bulk} value expresses by how much $^hE/^lE$ is smaller (negative values) or larger (positive

208   values) in the average of freshly formed products compared to the substrate from which they

209   are formed. Calculation of product carbon isotope fractionation trends and substrate AKIEs is

210   indicated in the SI. Compound-average ε_{bulk} values can be calculated using a modified form of

211   the Rayleigh distillation equation:

212

213     $\ln\frac{R_t}{R_0} = \ln\left(\frac{\delta\ ^hE_t + 1}{\delta\ ^hE_0 + 1}\right) = \varepsilon_{bulk} \cdot \ln f$ (3)

214

215   where R_t and R_0 are the current and initial isotope ratios, respectively, and f is the remaining

216   fraction of the compound. This equation can also be applied to calculate the isotopic

217   fractionation of chlorine despite the higher natural abundance of ^{37}Cl compared to ^{13}C.[53] The

218   ε_{bulk} values were quantified by least square linear regression of eq. 3, without forcing the

219   regression to the origin,[54] and the uncertainty corresponds to the 95% confidence interval

220   (C.I.) derived from the standard deviation of the regression slope (Figure S1).

221

222   **RESULTS AND DISCUSSION**

223   **Concentration patterns and reaction kinetics**

224   The 1,1,1-TCA transformation extent was $\geq$ 90% in all experiments (Figure 1). Degradation

225   of 1,1,1-TCA by activated PS lasted approximately 7 hours ($t_{1/2}$ = 1.5 h). Neither products nor

226   intermediates were detected by headspace GC/MS analysis (mass scan range 50 – 300 m/z)

227   performed during the course of reaction. In addition, the good agreement between measured

228   and expected Cl^- concentrations (assuming complete dechlorination), indicated that formation

229   of chlorinated products was probably insignificant (Figure 1). Experiments of HY/DH lasted

230   37 days ($t_{1/2}$ = 8.0 d) and 1,1,1-TCA was transformed to 1,1-DCE (yield of ~27%, see SI) and

231   Ac in parallel reactions. Finally, experiments with Fe(0) lasted 29 hours (apparent $t_{1/2}$ = 9.9 h)

232   and yielded 1,1-DCA via hydrogenolysis. Non-chlorinated products such as C_4 compounds

233   (from radical coupling reaction) and ethane/ethene (α-elimination) have also been identified

234   in agreement with previous studies.[14, 16, 18, 19] Concentration patterns of 1,1-DCA, ethene and

235   ethane (Figure 1) agreed well with their formation in parallel reactions (Scheme 1b).[16, 18] A

236   previous reaction study indicated that reduction of ethene by Fe(0) was too slow to account

237   for the amount of produced ethane and postulated that ethene could be formed through

238   rearrangement of $H_3C - \ddot{C} - H$ carbenoid (a four electron reduction intermediate of 1,1,1-

239   TCA transformation to ethane) (Scheme S1).[16] Final concentrations of 1,1-DCA, ethene,

240   ethane and remaining 1,1,1-TCA reflected a mass balance deficit of approximately 48% (i.e.

241   mass amount necessary to maintain the system mass balance expressed as percent of the

242   initial mass). A high deficit was previously observed for 1,1,1-TCA reduction by FeS (79%[55]

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243   and ~94%[14, 56]) or bimetallic reductants (up to ~20%[16, 19]). The detected but not quantified C_4,

244   C_5 and C_6 species could explain the unclosed mass balance in our study. In addition to C_4-C_6

245   compounds, acetaldehyde[57, 58] could be a potential product but was not analyzed in our study.

246   Chlorine isotope patterns of 1,1,1-TCA

247   The $\delta^{37}Cl$ of 1,1,1-TCA showed a trend to more positive values during its transformation by

248   HY/DH or Fe(0) (Figure 2), which reflects an enrichment of 1,1,1-TCA in heavy isotope

249   (^{37}Cl, eq. 2) indicative of a normal isotope effect. In contrast, for reaction of 1,1,1-TCA with

250   PS, a constant $\delta^{37}Cl$ was observed. For HY/DH and Fe(0) pathways, chlorine isotope data

251   followed a Rayleigh trend ($r^2 = 0.997$, eq. 3, Figure S1) and resultant ε_{bulk}^{Cl} values were in a

252   similar range, -4.7±0.1 and -5.2±0.2‰ for HY/DH and Fe(0), respectively (Figure 2).

253   Carbon isotope patterns for substrate and products

254   For all reactions investigated, an enrichment of 1,1,1-TCA in ^{13}C was observed indicative of a

255   normal isotope effect (Figure 2). The $\delta^{13}C$ of 1,1,1-TCA in the controls of all experiments (-

256   26.5±0.3‰) did not change significantly from the initial value ($\delta^{13}C_0$ = -26.3±0.1‰). The

257   carbon isotope composition of 1,1,1-TCA followed a Rayleigh trend ($r^2 \geq 0.96$, eq. 3, Figure

258   S1) with ε_{bulk}^{C} values ranging from -1.6‰ (HY/DH) to -7.8‰ (Fe(0)) (Figure 2). The

259   products analyzed were initially depleted in ^{13}C compared to the initial value of the substrate,

260   in agreement with the normal isotope effect. The $\delta^{13}C$ of the products also shifted towards

261   more positive values during the course of reaction reflecting the substrate enrichment in ^{13}C

262   (Figure 2).

263   For reaction with heat-activated PS, an ε_{bulk}^{C} of -4.0±0.2‰ was determined (Figure 2) but no

264   products were recovered for isotope analysis. The obtained carbon isotope enrichment factor

265   was smaller than the one reported for base-activated PS reaction (ε_{bulk}^{C} = -7.0±0.2‰) in a

266   recent study.[21] For HY/DH, the relatively small ε_{bulk}^{C} of -1.6±0.2‰ was consistent with the

267   small depletion in ^{13}C observed for 1,1-DCE. The largest carbon isotope enrichment factor

268   (ε_{bulk}^{C} = -7.8±0.4‰) was obtained for reduction by Fe(0), which agreed with the stronger

269   depletion in ^{13}C of some of the products (Figure 2). The α-elimination products (ethene and

270   ethane) showed a similar depletion in ^{13}C relative to the substrate, but a much larger depletion

271   in ^{13}C was observed for the hydrogenolysis product (1,1-DCA). The observed isotope pattern

272   with constant discrimination between products was indicative of product formation in parallel

273   pathways. In addition, the significant isotope discrimination between hydrogenolysis and α-

274   elimination products (about 9‰, Figure 2E) was consistent with the formation of a common

275   radical intermediate in the first reaction step (Scheme 1b). The reason is that different bonds

276   are broken or formed in subsequent reactions of the common intermediate (Scheme S1),

277   which are likely associated with different isotope effects. Interestingly, the product isotope

278   pattern for 1,1,1-TCA is different than that observed during reduction of TCE by Fe(0) in

279   previous studies.[46, 59] Contrary to reduction of 1,1,1-TCA, ethene/ethane produced from TCE

280   were constantly depleted in ^{13}C (about 10‰) compared to the hydrogenolysis product (cis-

281   dichloroethylene). The difference between the product isotope patterns of TCE and 1,1,1-

282   TCA could be due to distinct reaction mechanisms leading to formation of ethene/ethane. The

283   occurrence of distinct mechanisms is supported by previous studies indicating that production

284   of ethene/ethane from TCE mainly proceeds via β-dichloroelimination to chloroacetylene.[46, 60]

285   For the Fe(0) pathway, an ε_{bulk}^{C} of -13.6±0.5‰ from a previous study is available for

286   comparison.[36] This value is larger than the one determined in our study (-7.8±0.4‰),

287   however, significant variation ranging from -10.3 to -14.0‰ has also been recently observed

288   during reduction of 1,1,1-TCA by biotically mediated FeS formation in microcosm

289   experiments.[15] Similarly, high variability of ε_{bulk}^{C} has been reported for reductive

290   dechlorination of chlorinated ethenes by Fe(0). For instance, a range from -6.9 to -20.1‰ has

291 been observed for vinyl chloride (VC) where, in contrast to polychlorinated ethenes, only

292 hydrogenolysis is possible (see ref[46] and references herein). Such variability could be

293 explained by the presence of preceding rate-limiting steps.[61] If dehalogenation (isotope

294 fractionating step) becomes fast due to a high reactivity of the metal or substrate, preceding

295 steps such as the formation of organohalide-metal complexes may become rate-limiting. In

296 this case, if preceding (rate-limiting) steps exhibit small or no isotope fractionation, the

297 observable isotope effect will be smaller (i.e. masked) than the intrinsic isotope effect.[30, 61]

298 It is important to note that for cleavage of a C-Cl bond, primary isotope effects for carbon are

299 generally expected to be higher than for chlorine because carbon has a larger relative mass

300 difference between its heavy and light isotopes (i.e. 8.3% mass difference between $m_{12C} = 12$

301 and $m_{13C} = 13$ versus 5.7% mass difference between $m_{35Cl} = 35$ and $m_{37Cl} = 37$, relative to the

302 mass of the respective light isotope).[49] However, for reasons discussed in more detail below,

303 for HY/DH the isotope fractionation for chlorine (-4.7±0.1‰) was unexpectedly larger than

304 for carbon (-1.6±0.2‰), and in a similar range for reduction by Fe(0) (-5.2±0.2 and -

305 7.8±0.4‰, respectively).

Dual C-Cl isotope approach to differentiate degradation pathways

307 Carbon and chlorine δ isotope values of 1,1,1-TCA from the three investigated reaction

308 pathways were compared in a dual isotope plot. All reactions showed linear trends with

309 strongly different slopes (Figure 3). For reaction with PS, a vertical trend was obtained due to

310 the absence of chlorine isotope fractionation; whereas for HY/DH and Fe(0) reactions,

311 different slopes ($\Lambda = \Delta\delta^{13}C/\Delta\delta^{37}Cl \approx \varepsilon_{bulk}^{C}/\varepsilon_{bulk}^{Cl}$) were observed as a result of the difference

312 in their ε_{bulk}^{C} values (-1.6±0.2 and -7.8±0.4‰, respectively) compared to the similar ε_{bulk}^{Cl} (-

313 4.7±0.1 and -5.2±0.2‰, respectively). The clearly distinct dual C-Cl isotope trends for the

314 three reactions opens the possibility of a dual isotope approach to identify the different abiotic

315 degradation pathways of 1,1,1-TCA in the field. The large difference between trends also

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316　enables to estimate the proportion of two competing pathways based on the resultant slope,[62,]

317　[63] assuming simultaneous activity with a constant ratio between both pathway rates. Even if

318　ε^C_{bulk} values are significantly different for distinct reactions, a single element approach is not

319　sufficient to distinguish degradation pathways under field conditions. The reason is that a

320　certain extent of observable carbon isotope fractionation in the field (i.e $\Delta\delta^{13}C$) could have

321　been caused by a strongly isotope fractionating reaction that has proceeded little, or a weakly

322　isotope fractionating reaction that has proceeded further. Only the analysis of a second

323　element was possible to resolve this issue.

324　**Mechanistic insights**

325　The dual isotope trend observed for reaction of 1,1,1-TCA with PS, with constant $\delta^{37}Cl$

326　values, suggests that no cleavage of C-Cl occurs at the first reaction step. This result provides

327　a direct evidence of oxidative C-H bond cleavage in the first reaction step (Scheme 1c),

328　confirming 1,1,1-TCA degradation via H abstraction as proposed by a recent reaction study.[25]

329　The calculated ^{13}C-AKIE in our study (1.0081±0.0002, Table S2) was also consistent with the

330　typical ^{13}C-KIE for oxidative C-H bond cleavage (1.01 – 1.03).[49] The difference between the

331　ε^C_{bulk} value determined in our study (-4.0±0.2‰) and the value obtained for 1,1,1-TCA

332　reaction with base-activated PS (-7.0±0.2‰)[21] might be due to different underlying reaction

333　mechanisms. However, conclusion cannot be drawn as only carbon isotope ratios were

334　measured in the previous study with base-activated PS,[21] highlighting the benefit of using a

335　dual C-Cl isotope approach.

336　For the HY/DH and Fe(0) reaction pathways, distinctly different slopes were obtained

337　although both pathways involve cleavage of a C-Cl bond in their initial reaction step (Scheme

338　1a-b). To address this question, a closer look at the two pathways is necessary. For HY/DH,

339　the reaction could proceed via formation of a common carbocation intermediate (^{13}C-AKIE =

340　1.0033±0.0004, Table S2) or via two parallel reactions starting from the substrate (S_N2 vs. E2

341 mechanisms). If the latter case was true, the S_N2 reaction would dominate the isotope

342 fractionation of the substrate according with the product distribution (the yield of the Ac by

343 nucleophilic substitution was approximately 73%, Figure 1). However, considering the

344 usually large ^{13}C-KIE associated with S_N2 reaction (typical range from 1.03 to 1.09)[49] this

345 mechanism is less plausible compared to a S_N1 (typical range from 1.00 to 1.03)[49] (see SI).

346 Degradation of 1,1,1-TCA via formation of a common carbocation intermediate is supported

347 by previous reaction studies[11, 13] showing that variation of reaction conditions (i.e. T and pH)

348 did not cause significant changes in the product distribution and transformation rate,

349 respectively. Variation of reaction conditions as a means to induce changes in the product

350 distribution is a commonly used approach to obtain insight about branching points. However,

351 as it can be difficult to observe significant variation of the product ratio with varying reaction

352 conditions, sometimes it is not possible to draw sound conclusions using this approach.[12, 64]

353 Therefore, the isotope results show that complementary evidence could be obtained by

354 comparing determined AKIEs with expected KIEs (see SI). The resultant chlorine isotope

355 effect was large (^{37}Cl-AKIE = 1.0145±0.0003) relative to the carbon (^{13}C-AKIE =

356 1.0033±0.0004), which is not expected if isotope fractionation was caused only by primary

357 isotope effects associated with cleavage of a C-Cl bond (see above). Apparent KIEs (Table

358 S2) were calculated assuming that the first (isotope fractionating) step was only associated

359 with primary isotope effects and,[49] therefore, α-secondary ^{37}Cl-KIEs are very likely to

360 contribute. Similarly, for reduction by Fe(0), estimated AKIEs for C-Cl bond cleavage by

361 SET exhibited similar values, 1.0158±0.0008 (^{13}C-AKIE) and 1.0160±0.0006 (^{37}Cl-AKIE),

362 suggesting that α-secondary chlorine isotope effects also play a role. In summary, different

363 magnitudes of secondary chlorine isotope effects associated with the HY/DH and Fe(0)

364 reaction mechanisms could at least be partly responsible for the different dual C-Cl isotope

365 slopes. The occurrence of α-secondary ^{37}Cl-KIEs is supported by significant secondary

16

366 chlorine isotope effects measured experimentally during 1,2-dichloroethane (1,2-DCA)

367 aerobic biodegradation in a recent study.[37]

Environmental significance

369 Subsurface contamination by chlorinated ethanes is one of the major ecological problems and

370 the assessment of their fate in groundwater is a complex task. The distinctly different dual

371 isotope trends shows the feasibility of this approach for 1,1,1-TCA abiotic degradation

372 pathways differentiation in the field (Figure 3). The large difference of slopes (Λ) between the

373 abiotic reductive (Fe(0)) and oxidative (PS) pathways suggest that aerobic and anaerobic

374 biodegradation of 1,1,1-TCA could be also differentiated using this approach, provided that

375 these different isotope trends can be confirmed in future biodegradation studies. Aerobic and

376 anaerobic biotransformation of 1,1,1-TCA to 2,2,2-trichloroethanol (via C-H bond

377 oxidation)[65, 66] and to 1,1-DCA (via C-Cl bond cleavage),[6] respectively, has been well

378 documented in previous studies.

379 In addition, the product carbon isotope pattern obtained for 1,1,1-TCA reduction by Fe(0)

380 could be used to distinguish between biotic and abiotic reductive dechlorination. While biotic

381 sequential reductive dechlorination (i.e. 1,1,1-TCA $\rightarrow$ 1,1-DCA $\rightarrow$ CA $\rightarrow$ ethane) would

382 produce ethane initially depleted in ^{13}C relative to 1,1-DCA, the opposite was observed for

383 reaction with Fe(0). However, although biodegradation of CA to ethane has been reported,[67]

384 there is currently a lack of comprehensive experimental evidence for complete anaerobic

385 dechlorination of 1,1,1-TCA via hydrogenolysis.[3]

386 One of the main applications of CSIA to field studies is the estimation of contaminant

387 degradation extent using the Rayleigh equation.[68, 69] For this purpose, knowledge of the 1,1,1-

388 TCA degradation pathway at the site and its corresponding ε_{bulk}^{C} and/or ε_{bulk}^{Cl} values are

389 necessary. However, in contrast to chlorinated ethenes, reported ε_{bulk} values for chlorinated

390 ethanes are scarce in the literature. The new isotope fractionation values of 1,1,1-TCA

17

391   determined for HY/DH, oxidation by heat-activated PS (both ε_{bulk}^{C} and ε_{bulk}^{Cl}) and reduction

392   by Fe(0) (ε_{bulk}^{Cl}) increase the possibility of using CSIA in sites polluted by 1,1,1-TCA. In

393   addition, higher absolute ε_{bulk} values lead to estimates with a minor uncertainty[68] and,

394   therefore, for HY/DH chlorine isotope ratios (ε_{bulk}^{Cl} = -4.7±0.1‰) could be used instead of

395   carbon (ε_{bulk}^{C} = -1.6±0.2‰) to assess 1,1,1-TCA transformation in the field. This study

396   demonstrates that abiotic 1,1,1-TCA transformation processes can unambiguously be

397   identified and evaluated using a dual C-Cl isotope approach.

398

399   ASSOCIATED CONTENT

400   **Supporting Information**

401   Further information about experiment set up details, analytical methods, reaction kinetics,

402   Rayleigh isotope plots (Figure S1), calculation of product carbon isotope fractionation trends

403   and AKIEs (Table S1 and S2), estimation of ^{13}C-AKIE for HY/DH of 1,1,1-TCA via S_N2/E2

404   mechanisms and, degradation pathway for reaction of 1,1,1-TCA with Fe(0) (Scheme S1) is

405   available. This material is available free of charge via the Internet at http://pubs.acs.org.

406   AUTHOR INFORMATION

407   **Corresponding Author***

408   Jordi Palau

409   Centre d'Hydrogéologie et de Géothermie, Université de Neuchâtel, Rue Emile-Argand 11,

410   CH-2000 Neuchâtel, Switzerland

411   E-mail: jordi.palau@ub.edu

412   **Author Contributions**

413   The manuscript was written through contributions of all authors. All authors have given

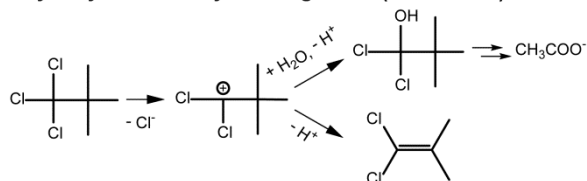414   approval to the final version of the manuscript.

415   ACKNOWLEDGEMENTS
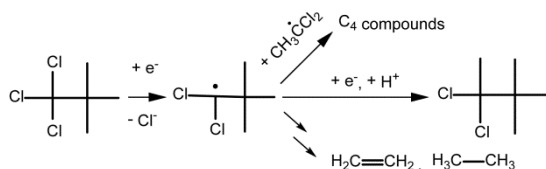
420

421 **Scheme 1.** Proposed abiotic degradation pathways for 1,1,1-TCA (dots in structural formulas
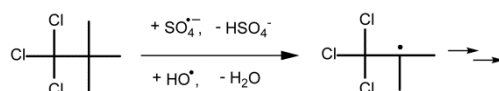422 represent unpaired electrons).
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a. Hydrolysis and dehydrohalegantion (S$_N$1 and E1)

b. Reduction by Fe(0) (SET)

c. Reaction with heat-activated PS (Oxidative C-H bond cleavage)
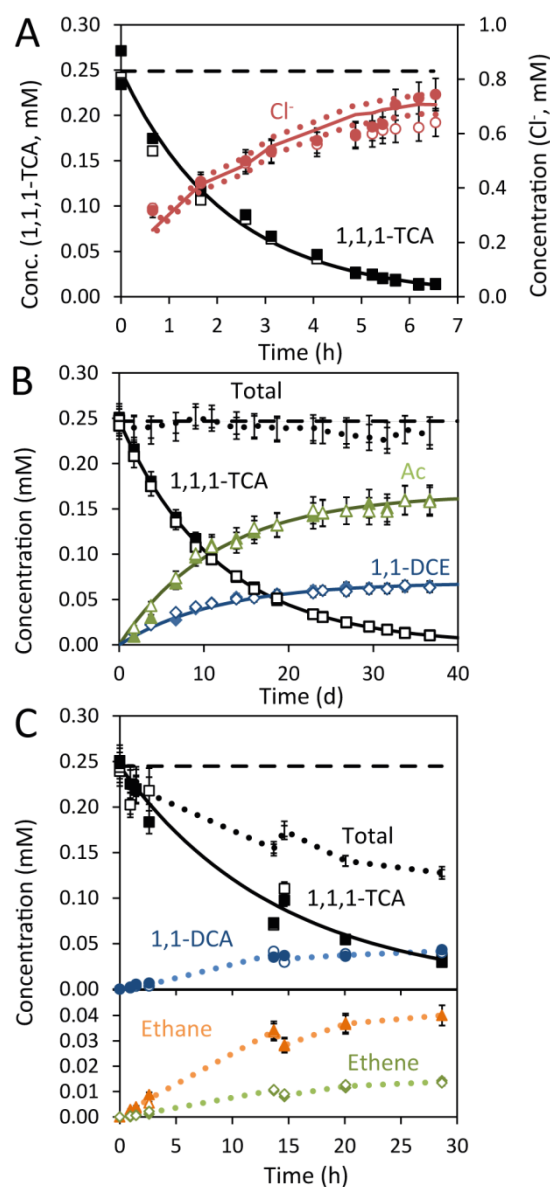
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Figure 1. Concentration of 1,1,1-TCA and target products for (A) reaction with PS, (B) HY/DH and (C) reduction by Fe(0) experiments. Data from duplicate experiments were combined (i.e. filled and empty symbols). The total (error bars without symbol marks in panels B and C), the average 1,1,1-TCA concentration in the controls (- - -, see values in the SI) and the pseudo-first order fitting of 1,1,1-TCA, 1,1-DCE and Ac concentration data (——, eqs. S1-S3) are indicated. In panel A, the average (——) and the range (dotted lines) of theoretical Cl⁻ concentrations from duplicate experiments are shown. Error bars correspond to the total relative uncertainties of $\leq 8\%$ (for 1,1,1-TCA, 1,1-DCE and 1,1-DCA), $\leq 10\%$ (for ethene and ethane) and 8% (for Ac and Cl⁻). In some cases error bars are smaller than the symbols.
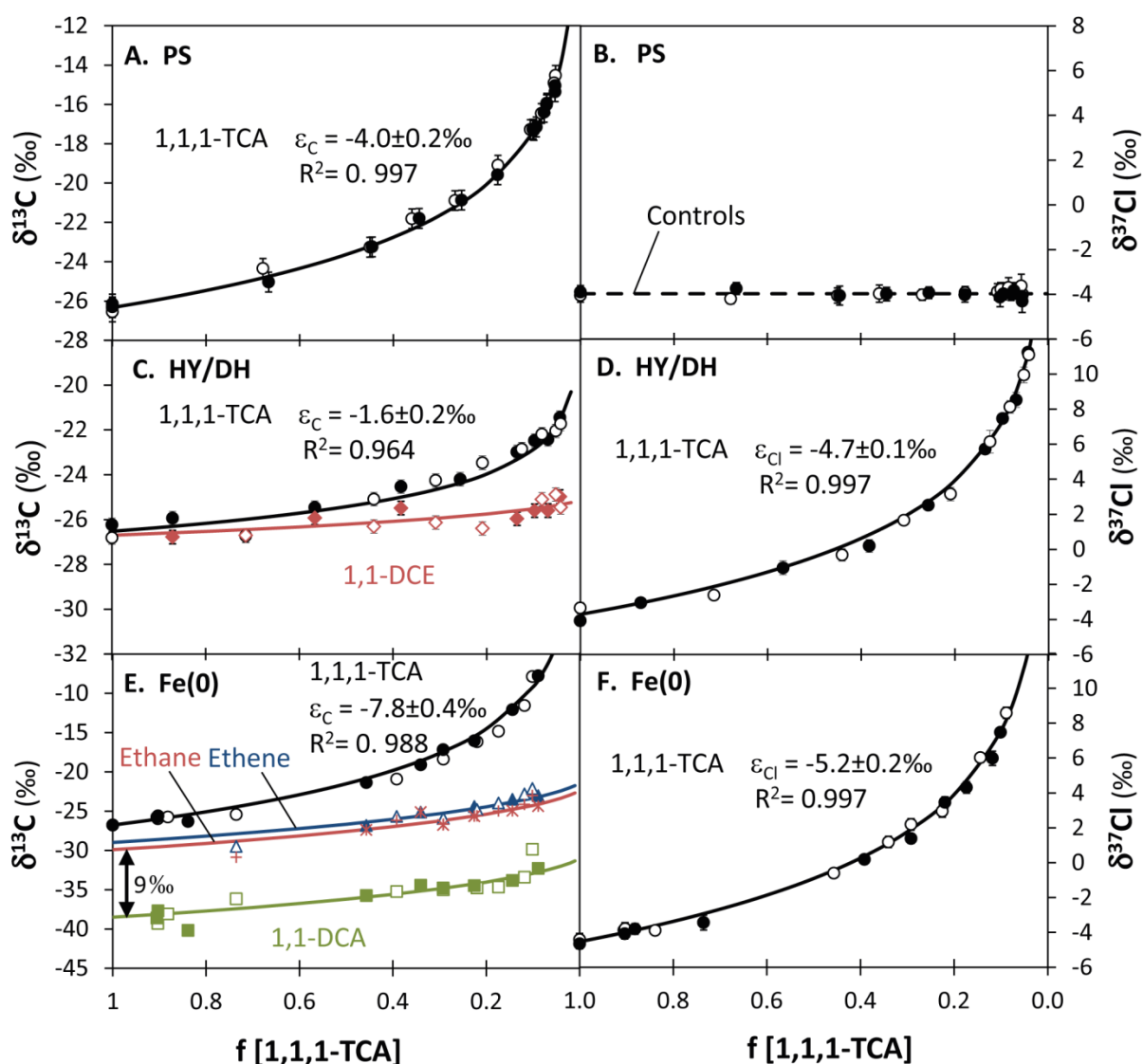
Figure 2. Isotopic composition of the substrate ($\delta^{13}C$ and $\delta^{37}Cl$) and products ($\delta^{13}C$) for reaction of 1,1,1-TCA with PS (A, B), HY/DH (C, D) and reduction by Fe(0) (E, F). Data from duplicate experiments were combined and symbols are as follows: 1,1,1-TCA (circles), 1,1-DCE (diamonds), 1,1-DCA (squares), ethene (triangles) and ethane (crosses). The uncertainty of ε_{bulk} values corresponds to the 95% confidence interval. The average $\delta^{37}Cl$ of 1,1,1-TCA in the controls (- - -, panel B) and models fit (——) to isotope data from the substrate (eq. 3) and products (eq. S4) are shown.
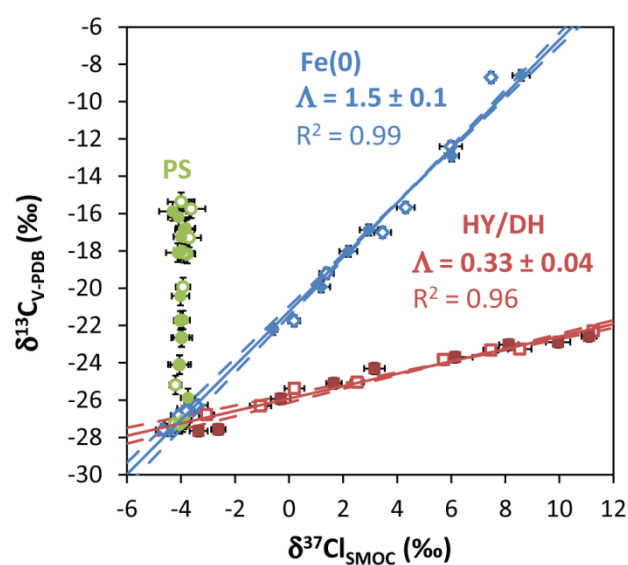
Figure 3. Dual C-Cl isotope plots during transformation of 1,1,1-TCA in the investigated experimental systems. Data from duplicate experiments were combined, i.e. empty and filled symbols: red squares for HY/DH, blue rhombus for reduction by Fe(0) and green circles for reaction with PS. Λ values ($\pm$95% C.I.) are given by the slope of the linear regressions (——) and the lines (- - -) correspond to the 95% C.I.

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